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THE PEACH REPLANT PROBLEM IN ONTARIO

III. AMYGDALIN CONTENT OF PEACH TREE TISSUES[1]

BY G. M. WARD[2] AND A. B. DURKEE[3]

Abstract

A chemical analysis of various tissues of the peach tree for their content of the cyanogenetic glycoside amygdalin has shown that the amount varies from none in the woody tissues to more than 50 milligrams per gram of dry tissue in some roots. The highest concentrations are found in the bark of the roots. Factors such as variety and season affect the amygdalin content.

Introduction

The nature and extent of the peach replant problem in Ontario have been described by Koch (3). Patrick (4) has demonstrated that toxic substances are formed on the microbial decomposition of peach root residues and has emphasized the leading role played by amygdalin. This report gives the amygdalin content of roots, branches, leaves, and buds of peach seedlings and trees.

Material and Methods

All samples used in this investigation came from the peach orchards of the Science Service Laboratory and the Experimental Station at Harrow, Ontario, in 1954 and 1955. Lovell is the commonest rootstock and most of the samples were from this variety. Analyses were made of other varieties also, including the somewhat resistant Yunnan and Shalil (2). The trees were of different ages; data were obtained for the variation between individual trees and between seasons.

Two-year-old seedlings of the 1954 sampling were separated into four samples each as shown in Table I. Three-year-old seedlings of the 1955 sampling were subdivided still further as indicated in Table II. The examination of other trees was confined to root tissues. In some instances the bark was removed from the large roots and analyzed separately from the wood. All samples were cleaned, air-dried, and ground before analysis.

[1]*Manuscript received February 28, 1956.*
Contribution No. 311 from the Chemistry Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

[2]*Senior Chemist.*
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[The May number of this journal (Can. J. Botany, 34 : 321–418. 1956) was issued May 17, 1956.]

The analysis for amygdalin was made by the steam distillation method for cyanogenetic glucosides (1). In practice it was found that leaf tissue did not require pretreatment with water to obtain complete release of the hydrogen cyanide. With all other tissues only a small fraction of the amygdalin was hydrolyzed unless autolysis was included. The amygdalin content was calculated on the basis of 1 mole $AgNO_3$ = 1 mole HCN = 1 mole amygdalin. Calculations were based on the assumption that all the HCN came from amygdalin.

Results

Table I records the amygdalin content of tissue samples from two seedling trees taken on June 25 and three seedling trees taken on October 22 from the same location in the same year. Table II shows the amygdalin content of four replicate trees sampled in the second year of the experiment. Table III gives the amygdalin content of root tissues from trees of several peach varieties and varietal combinations. The results are expressed as milligrams of amygdalin per gram of dry weight of tissue.

A considerable variability was found in the amygdalin content of corresponding tissues from different trees. For example, Tree C (Table I) sampled in October 1954 had much less amygdalin than either of the other trees sampled on the same date. A part of the variation between replicate samples may be explained by the observation that a strong odor of cyanide and benzaldehyde was always present at the time of sampling, indicating a loss

TABLE I

AMYGDALIN CONTENT OF TWO-YEAR-OLD LOVELL PEACH SEEDLINGS

		Amygdalin (mgm./gm. dry weight)			
Date of sampling	Tree No.	Leaves	Stem, branches, twigs	Large roots	Small roots
June 25, 1954	A	7.4	4.9	41.5	53.8
June 25, 1954	B	7.1	4.7	39.8	36.6
October 22, 1954	C	13.0	9.3	15.5	22.8
October 22, 1954	D	18.3	9.3	32.8	37.8
October 22, 1954	E	16.9	4.8	21.4	26.1

TABLE II

AMYGDALIN CONTENT OF THREE-YEAR-OLD LOVELL PEACH SEEDLINGS
(Date of sampling November 24, 1955)

	Amygdalin (mgm./gm. dry weight)									
Tree No.	Large roots	Medium roots	Small roots	Root bark	Root wood	Small branches	Buds	Large branches	Branch bark	Branch wood
F	12.3	39.7	37.4	43.2	3.4	11.5		0	5.2	0.6
G	20.7	35.7	48.8	28.8	2.9	14.4		0		
H	21.3	43.7	39.7	30.5	10.4	5.2	0	0.6		
I	20.7	38.0	53.5	43.2	15.6	16.6		0.5		

TABLE III

AMYGDALIN CONTENT OF PEACH TREE ROOTS

Date of sampling	Tree No.	Variety	Age (yr.)	Amygdalin (mgm./gm. dry weight)			
				Large roots	Small roots	Root bark	Root wood
October 22, 1954	J	Lovell	2			56.6	3.8
November 5, 1954	K	Lovell	8		16.3	27.3	0.0
November 20, 1954	L	Yunnan	2			19.6	1.8
November 20, 1954	M	Shalil	2			24.0	Trace
November 24, 1955	N	Elberta on Lovell	6	26.4	31.0	34.5	5.2
November 24, 1955	O	Elberta on Lovell	6	31.6	27.6	44.8	7.5
November 24, 1955	P	Golden Jubilee on Lovell	6	28.2	32.2	38.5	7.5
November 24, 1955	Q	Early Hale Haven on Lovell	6	32.8	33.4	48.9	8.6

of amygdalin. It is apparent that mechanical injury of the tissues resulted in enzymic hydrolysis of the glycoside. The amount lost cannot be accurately estimated but might be sufficient to account for some of the lack of uniformity. The trees sampled in the fall (Table I) had a higher concentration of amygdalin in the above-ground portions and a lower concentration of amygdalin in the roots than those sampled in the spring.

The highest percentage of amygdalin was in the roots, where it was found to be concentrated in the root bark. The small amounts found in some samples of root wood may have been due to contamination with small amounts of bark or it may have been due to diffusion of some of the glycoside into adjacent areas of the wood. From the data in Table III it is difficult to detect any definite pattern of varietal difference in amygdalin content other than the observation that root bark of the varieties Yunnan and Shalil had considerably less than that of other varieties.

The results provide additional evidence for Patrick's hypothesis (4) for the cause of peach replant failure by demonstrating that peach root bark is the most probable source of the toxic substances which have been shown to produce root damage in the laboratory similar to that found with the occurrence of the disorder in the orchard. It is not at present possible to interpret the resistance of the Yunnan and Shalil varieties of peach to replant failure as a function of amygdalin content on the basis of these analyses and in reference to the root residue theory. The analyses only provide further evidence for the complex nature of the replant disorder. An explanation will probably become apparent when all the conditions governing amygdalin hydrolysis in the soil are more clearly understood.

Acknowledgments

Acknowledgment is expressed to Dr. L. W. Koch of the Science Service Laboratory, Harrow, Ontario, for providing the opportunity to conduct this survey, to Dr. Z. A. Patrick for assistance in planning and for collecting and preparing samples of tissue, and to other members of the Science Service Peach Replant Committee for helpful suggestions.

References

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official methods of analysis of the A.O.A.C. 7th ed. Washington. 1950.
2. HARRISON, T. B. Horticultural investigations on the peach replant problem. Progr. Rept. No. 3, Peach Replant Committee, Can. Dept. Agr. April, 1955. (Unpublished data.)
3. KOCH, L. W. The peach replant problem in Ontario. I. Symptomatology and distribution. Can. J. Botany, 33 : 450-460. 1955.
4. PATRICK, Z. A. The peach replant problem in Ontario. II. Toxic substances from microbial decomposition products of peach root residues. Can. J. Botany, 33 : 461-486. 1955.

METABOLISM OF C^{14} AMINO ACIDS AND AMIDES IN DETACHED LEAVES[1]

By C. D. Nelson[2] and G. Krotkov

Abstract

Detached broad bean leaves were placed with their petioles in 0.01 *M* ammonium nitrate and allowed to carry on photosynthesis in $C^{14}O_2$ for various periods from 12 to 125 min. The radioactivities of the various amino acids formed from $C^{14}O_2$ were determined. In addition, these amino acids were degraded by decarboxylation with ninhydrin. From the specific activity data it was concluded that the amino acid closest to the site of carbon dioxide fixation in photosynthesis was alanine, followed by aspartic and glutamic acids, with the amides farthest removed. From the intramolecular distribution of label it was concluded that asparagine and glutamine were formed from their corresponding amino acids. The labelling in aspartic and glutamic acids was not consistent with the view that these two amino acids are formed from their corresponding α-keto acids produced by operation of the conventional tricarboxylic acid cycle. A C_2 plus C_2 condensation is postulated for the formation of aspartic acid. A shift in the double bond in the aconitase reaction of the tricarboxylic acid cycle would account for the observed labelling in glutamic acid. When acetate-1-C^{14} was fed to detached broad bean leaves in the light or dark, the distribution of label in glutamic acid supported the suggestion that there is such a shift in the double bond in the aconitase reaction. Sodium arsenite, infiltrated into tobacco leaves, inhibited the biosynthesis of asparagine but not that of glutamine.

Introduction

Several comprehensive studies of the over-all metabolism of excised leaves and seedlings have been carried out in an attempt to co-ordinate changes in sugars, organic acids, and nitrogenous compounds. Yemm (20) studied the effect on amide accumulation of a wide variety of carbohydrate concentrations by harvesting field grown barley leaves at different times during the growing season. He concluded that glutamine synthesis was dependent upon organic acid precursors. Vickery *et al.* (17) made comprehensive analyses of the changes in excised tobacco leaves during water culture in either light or dark. They concluded that the carbohydrate concentration controlled the amount of keto acid precursors available for amide synthesis; when carbohydrate concentration was high α-ketoglutarate was available for glutamine synthesis, and when carbohydrate was low oxalacetate was available for asparagine synthesis. Meiss (9) has extended this work of Vickery's to germinating lupine seedlings. He concluded that organic acid analysis, as traditionally carried out, contributed little to the understanding of carbon precursors of nitrogen compounds. Bidwell, Krotkov, and Reed (2) have used isotopic carbon to show that the carbon chain of glutamine is in equilibrium with carbohydrates in Swiss chard leaves.

[1]*Manuscript received March 12, 1956.*
Contribution from the Department of Biology, Queen's University, Kingston, Ontario, Canada, with financial support from the National Research Council of Canada.
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Elliot (5) first demonstrated glutamine synthesis in cell-free plant systems with a preparation from *Lupinus* seedlings. He showed that the mechanism is a direct amidation of glutamate in the presence of adenosine triphosphate. Recently Webster and Varner (19) have found a system in lupines and wheat germ for the synthesis of asparagine analogous to that of glutamine. On the other hand, Meister and Fraser (10) have found that the amination of α-ketosuccinamic acid to asparagine is catalyzed by an enzyme in rat liver preparations. Unfortunately, the synthesis of α-ketosuccinamate has not been demonstrated and it is still questionable whether this reaction participates in asparagine synthesis in a plant cell.

The controversy over the structure of asparagine and glutamine has pointed out differences in chemical behavior that cannot be accounted for on the basis that the amides are chemically homologous compounds (14). Since these differences in chemical behavior exist it is reasonable to assume that the metabolism of the amides *in vivo* is also different. The following experiments were undertaken to elucidate the metabolic pathways for the biosynthesis of the amides and their corresponding amino acids.

Materials and Methods

Tobacco leaves, *Nicotiana tabacum*, variety Connecticut seed leaf, and broad bean leaves, *Vicia faba*, were used as experimental material. Tobacco was grown in pots, and broad bean in flats containing loamy soil. Both plants were kept in the greenhouse under fluorescent lights for 16 hr. daily.

Sodium acetate-1-C^{14} was prepared by the authors in the laboratory of W. H. Stevens,* Atomic Energy of Canada Ltd., Chalk River, Ontario.

Infiltration and Administration of C^{14}

Broad bean leaves were detached from mature plants and their petioles immersed in 0.01 *M* ammonium nitrate. Leaves were then transferred to an 8 liter desiccator containing $C^{14}O_2$. The desiccator was illuminated with incandescent lights to give an intensity of 800 ft-c. at the leaf surface.

Sodium acetate-1-C^{14} was fed through the petiole as a 0.04 *M* solution adjusted to pH 5.5 with HCl. Specific activity of the acetate was 116 μc. per mM. Leaves were illuminated and put in the breeze of a fan for one hour during which time they took up about 0.75 ml. of solution per gram fresh weight of leaf. Subsequently, the leaves were placed in tap water and put in the light or the dark.

A 10^{-3} *M* solution of sodium arsenite was infiltrated into mature tobacco leaves as suggested by Kursanov (8) and for a control another leaf was infiltrated with water. After the leaves were partially dried in the light in a stream of air to remove the excess water, they were placed in the desiccator for $C^{14}O_2$ feeding.

All leaves were killed by boiling for one minute in 50% ethanol and extracted in cold ethanol. Two additional extractions with warm ethanol removed all the soluble amino compounds without hydrolysis of glutamine.

The authors wish to thank Dr. Stevens for his help in the preparation of this compound.

Chromatography

Alcoholic extracts were evaporated to dryness *in vacuo* at 35° C. and redissolved in small amounts of water for chromatography. Two-dimensional descending paper chromatography was used; the method followed was similar to that of Thompson and Steward (15) except that *n*-butanol – 95% ethanol – water in the proportions 53 : 32 : 16 was the second chromatographic solvent instead of collidine–lutidine. Quantitative determinations of sucrose on chromatograms were made by eluting spots, which were located by radioautography, and making colorimetric determinations on aliquots with anthrone reagent (11). Quantitative determinations of amino acids and amides were made using the method of Connell, Dixon, and Hanes (4). Radioactivities of chromatographically isolated compounds were determined after aliquots from eluted spots were plated on aluminum planchets. The compounds were identified by colorimetric tests, relative positions on the paper, and R_f values. In addition the amino acids were co-chromatographed with authentic samples using acetone–water, 4 : 1, and *n*-butanol – acetic acid – water, 9 : 1 : 4, as solvents.

Degradation

Amino acids and amides were decarboxylated with ninhydrin. The reaction mixture for degradation consisted of from 0.25 to 0.45 mM. of the compound to be degraded in 80 ml. of water; 0.3 gm. of ninhydrin in 5 ml. of methyl cellosolve; 5 ml. of 0.2 *M* citrate buffer, pH 5; and 16 mgm. of stannous chloride. After heating for 20 min. on a boiling water bath, from 98 to 101% of the theoretical yield of carbon dioxide was produced for all amino acids and amides degraded. Asparagine was degraded as such, yielding one mole of carbon dioxide per mole of amide, and also after hydrolysis to aspartic acid, yielding two moles of carbon dioxide per mole of amino acid. Average radioactivity of asparagine and glutamine carbon for the whole molecule was determined by plating a small amount of each amide and counting it as such. Radioactivity in the carboxyl carbon was determined by plating and counting, as barium carbonate, the carbon dioxide obtained from the decarboxylation with ninhydrin. No correction for self scattering need be made when carbon is counted in these two forms with the methane flow counter (12).

Radioactivity incorporated into the 50% ethanol soluble and insoluble fractions of the leaves was determined after combustion to carbon dioxide by the method of Baker *et al.* (1) and counting as barium carbonate. In all cases samples were counted for the period of time necessary to give a statistical error of less than 4%.

Results

$C^{14}O_2$ as a Precursor of Amino Acids and Amides

In order to observe synthesis of amino acids and amides from CO_2 the following experiments were performed. Four broad bean leaves with their petioles immersed in 0.01 *M* ammonium nitrate were placed in the photochamber containing 20 mM. of $C^{14}O_2$ with specific activity of 0.52 mc. per mM.

A single leaf was pulled out of this chamber after periods of photosynthesis of 12, 30, 50, and 125 min. Each leaf was killed immediately by being dropped into boiling 50% ethanol. The amino acids, amides, and sucrose were isolated and the analyses made as described above.

Table I shows the progress for the incorporation of radioactivity into the 50% ethanol insoluble fraction. This fraction included the cell wall material, lipids, and proteins. After an initial lag period the incorporation rate increased linearly (between 12 and 50 min.) and then reached a more or less constant value.

Table II gives the concentration, total activity, and specific activity of amino acids, amides, and sucrose isolated from the broad bean leaves. The high total and specific activity of aspartic acid confirm the role of the acid as an active metabolite in these leaves. In addition, since activity appeared in aspartic acid and alanine before it appeared in glutamic acid or the amides, the former two compounds must be considered closer to the site (or sites) of carbon dioxide fixation than the latter compounds.

TABLE I

TOTAL RADIOACTIVITY IN THE 50% ETHANOL INSOLUBLE RESIDUE OF BROAD BEAN LEAVES AFTER VARIOUS PERIODS OF PHOTOSYNTHESIS

Time in $C^{14}O_2$, min.	Total activity, μc. per gm. fr. wt.
12	0.023
30	0.431
50	0.788
125	0.961

The concentration of asparagine was 50 to 100 times that of glutamine. However, the total activity incorporated into these two amides at the end of the experiment was about the same. The specific activity data show that both amides are farther removed from the carboxylation reactions of photosynthesis than aspartic acid, alanine, or sucrose.

The concentration of glutamic acid was not as constant as that of aspartic acid. However, the total activity incorporated into glutamic acid at the end of the experiment was about the same as that incorporated into aspartic acid. The specific activity of glutamic acid never reached the high value of aspartic acid, but it was higher than that of the amides.

Alanine concentration was low and variable. From the high total activity and the extremely high specific activity of alanine after 12 min. illumination, it is concluded that this amino acid is closer to carbon dioxide fixation than any other compound isolated. It is known that alanine may be formed from pyruvate which is close to phosphoglyceric acid, the first stable compound formed in photosynthesis (3). These data from broad bean leaves support this conclusion.

TABLE II

CONCENTRATION, TOTAL ACTIVITY, AND SPECIFIC ACTIVITY OF AMINO ACIDS, AMIDES, AND SUCROSE IN DETACHED BROAD BEAN LEAVES AFTER VARIOUS PERIODS OF PHOTOSYNTHESIS IN $C^{14}O_2$. PETIOLES WERE IMMERSED IN 0.01 *M* AMMONIUM NITRATE

	Time in $C^{14}O_2$, (min.)			
	12	30	50	125
Aspartic acid				
Concentration*	0.82	0.84	0.80	0.74
Total activity†	0.063	0.095	0.147	1.050
Spec. activity‡	1.62	2.35	3.83	29.45
Asparagine				
Concentration*	52.0	43.0	45.0	60.0
Total activity†	0.0	0.033	0.049	0.201
Spec. activity‡	0.0	0.017	0.025	0.071
Glutamic acid				
Concentration*	1.47	1.94	1.90	2.03
Total activity†	0.0	0.367	0.649	1.167
Spec. activity‡	0.0	3.16	5.68	9.60
Glutamine				
Concentration*	0.42	0.58	1.08	0.58
Total activity†	0.0	0.032	0.139	0.242
Spec. activity‡	0.0	0.90	2.15	6.95
Alanine				
Concentration*	0.70	0.96	1.18	0.88
Total activity†	0.326	1.117	1.80	2.02
Spec. activity‡	12.88	32.30	42.40	63.50
Sucrose				
Concentration*	7.38	7.77	9.05	17.60
Total activity†	0.67	21.65	27.20	92.40
Spec. activity‡	0.63	19.30	20.70	36.70

*μM. *per gm. fr. wt.*
†μc. *per gm. fr. wt.*
‡μc. *per mgm. C.*

Sucrose was higher in concentration than any of the amino acids and accumulated very large amounts of radioactivity. After 12 min. of photosynthesis, its concentration was 10 times that of alanine and its specific activity was about one-half of that of alanine. This is consistent with the formation of sucrose from phosphoglyceric acid via reverse glycolysis.

The data obtained by degradation of the amino acids are given in Table III. Since the C_1 of glutamic acid and glutamine were labelled to the same extent and since the specific activity of glutamic acid was always greater than that of its amide, it is concluded that glutamine was formed from glutamic acid. Similarly, since the C_1 plus C_4 of aspartic acid and asparagine were labelled to the same extent, asparagine must have been formed from aspartic acid.

The small amount of labelling in the C_1 of glutamic acid is not in agreement with the presently accepted scheme for the formation of its carbon skeleton. α-Ketoglutaric acid, formed in aerobic organisms by operation of the

TABLE III

DISTRIBUTION OF C^{14} IN AMINO ACIDS AND AMIDES IN DETACHED BROAD BEAN LEAVES AFTER VARIOUS PERIODS OF PHOTOSYNTHESIS IN $C^{14}O_2$

		Time in $C^{14}O_2$, min.			
		12	30	50	125
Aspartic acid	$C_1 + C_4$	39%	31%	28%	32%
	$C_2 + C_3$	61	69	72	68
Asparagine	C_1	0	0	3	9
	$C_2 + C_3 + C_4$	0	100	97	91
Asparagine*	$C_1 + C_4$	—	—	—	26
	$C_2 + C_3$	—	—	—	74
Glutamic acid	C_1	0	2	4	4
	$C_2 + C_3 + C_4 + C_5$	0	98	96	96
Glutamine	C_1	0	0	3	10
	$C_2 + C_3 + C_4 + C_5$	0	100	97	90
Alanine	C_1	34	34	33	34
	$C_2 + C_3$	66	66	67	66

**Degradation of aspartic acid derived from hydrolysis of asparagine.*

tricarboxylic acid cycle, is usually considered to be the immediate precursor of glutamic acid. In the absence of recycling, labelled carbon dioxide should give rise to labelling only in the C_1 of α-ketoglutarate. Recycling would not allow labelling in any other atom of α-ketoglutarate. Reversal of the cycle from oxalacetate through succinate to α-ketoglutarate would also introduce label from carbon dioxide into the C_1 of α-ketoglutarate and hence into the C_1 of glutamic acid and glutamine.

An assumption that aspartic acid is formed from a 4-carbon acid produced in the tricarboxylic acid cycle is not supported by the observed labelling in aspartic acid. After 125 min. the C_1 plus C_4 of aspartic acid contained 32% of the radioactivity in the molecule. Since asparagine was formed from aspartic acid, the relative distribution of C^{14} in these two compounds must be the same. Therefore, one concludes that 9% out of a total of 32% was in the C_1 of aspartic acid and 23% in the C_4 position. This unequal distribution of label in the C_1 and C_4 positions precludes any possibility of aspartic acid formation via a symmetrical intermediate such as succinate. Also the very high activity in C_2 plus C_3 of aspartic acid even after the shortest period of illumination indicated that aspartic acid was formed from some precursor other than an acid produced in the tricarboxylic acid cycle.

Since alanine had about one-third of its activity in the carboxyl group, this amino acid was probably evenly labelled. This is in agreement with the accepted mechanism of alanine formation from phosphoglyceric acid via pyruvate (6).

Sodium Acetate-1-C^{14} as Precursor of Amino Acids and Amides

The unusual labelling patterns of aspartic acid, glutamic acid, and their amides suggested that acetate may be the possible precursor. Therefore, it was decided to introduce sodium acetate-1-C^{14} into three broad bean leaves. Since the leaves would not take up solution through their petioles when placed in the dark, all three leaves were fed for one hour in the light. After introduction of acetate one leaf was killed and extracted immediately, while the other two leaves were put with their petioles in water, one in the dark and the other in the light. At the end of three hours these leaves were analyzed as described for the earlier experiment.

TABLE IV

INCORPORATION OF RADIOACTIVITY INTO THE 50% ETHANOL SOLUBLE AND INSOLUBLE FRACTIONS OF DETACHED BROAD BEAN LEAVES FED ACETATE-1-C^{14}

Leaf	Conditions	Total activity per leaf in 50% ethanol soluble fraction, μc.	Total activity per leaf in 50% ethanol insoluble fraction, μc.	Total incorporation per leaf, μc.
1	1 hr. light HAc	0.61 (80%)	0.15 (20%)	0.76 (100%)
2	1 hr. light HAc 3 hr. light H_2O	0.17 (60%)	0.11 (40%)	0.28 (100%)
3	1 hr. light HAc 3 hr. dark H_2O	0.60 (72%)	0.23 (28%)	0.83 (100%)

Table IV shows the results of the incorporation of radioactivity into the 50% ethanol soluble and insoluble fractions. Although the three leaves were of approximately the same weight it was observed that leaves 1 and 3 took up about the same amount of radioactivity while leaf 2 took up about three times less. The insoluble fraction included the lipids which accounts for the high incorporation, more than 20% of the total, into this fraction. A greater percentage of the acetate absorbed by the leaf went into the insoluble fraction in the light than in the dark.

Aspartic acid, glutamic acid, and glutamine were the only radioactive compounds that could be detected in the 50% ethanol soluble fractions of the three leaves. On the other hand, when $C^{14}O_2$ is fed, many compounds become radioactive in one to three hours. It is concluded that acetate was not oxidized to carbon dioxide before the label was incorporated into these amino acids. It should be noted that no radioactivity appeared in the alanine fraction although alanine was present in the leaf in a concentration of 0.32 μM. per gm. fresh weight.

Table V shows that in two cases out of three the concentration of glutamic acid was greater than either aspartic acid or glutamine, and glutamic acid accumulated from 2 to 10 times more radioactivity. If it is assumed that the

TABLE V

CONCENTRATION, TOTAL ACTIVITY, SPECIFIC ACTIVITY, AND DEGRADATION DATA FOR AMINO ACIDS IN DETACHED BROAD BEAN LEAVES FED ACETATE-1-C^{14}

Leaf	Conditions	Compound	Conc. μM. per gm. fr. wt. of leaf	Total activity, mμc. per gm. fr. wt. of leaf	Specific activity, mμc. per mgm. C	% of total activity in alpha carboxyl
1	1 hr. light HAc	Aspartic acid	1.59	7.2 ± 20%	—	—
		Glutamic acid	4.30	50.4 ± 4%	196.6	8%
		Glutamine	0.74	5.4 ± 20%	—	—
2	1 hr. light HAc	Aspartic acid	1.68	4.5 ± 20%	—	—
	3 hr. light H_2O	Glutamic acid	2.60	10.8 ± 4%	81.6	—
		Glutamine	0.76	5.4 ± 20%	—	—
3	1 hr. light HAc	Aspartic acid	2.88	7.2 ± 20%	—	—
	3 hr. dark H_2O	Glutamic acid	2.86	23.4 ± 4%	136.6	13%
		Glutamine	0.54	4.5 ± 20%	—	—

specific activity of glutamic acid was about the same in the three leaves after the one hour feeding period (i.e. 196.6 mμc. per mgm. C) then the glutamic acid pool was turned over more rapidly in the light (specific activity dropped to 8.6 mμc. per mgm. C) than in the dark (specific activity dropped to 136.6 mμc. per mgm. C).

The degradation data for glutamic acid show that up to 13% of the radioactivity in the molecule was in the C_1 position. If glutamic acid was formed from α-ketoglutarate through the operation of the tricarboxylic acid cycle, the C_1 of acetate should be incorporated into the C_5 of glutamic acid and not into the C_1. It is concluded that the tricarboxylic acid cycle cannot account for the formation of glutamic acid in broad bean.

Effect of Sodium Arsenite on the Biosynthesis of Amides in Detached Tobacco Leaves

Since sodium arsenite is known to be a general physiological inhibitor of synthetic reactions it was of interest to observe its effect on the biosynthesis of amides. Two mature tobacco leaves were infiltrated in water and sodium arsenite respectively. Both leaves were then fed $C^{14}O_2$, specific activity 1.91 μc. per mgm. C, for 48 hr. in the light. The results of leaf analysis are shown in Table VI.

The leaf infiltrated with water gained in fresh weight while that infiltrated with arsenite lost weight. The concentrations of sucrose and glutamine were lower in the poisoned leaf than in the control. No asparagine could be detected in the poisoned leaf. The specific activity data show that arsenite has relatively little effect on the incorporation of $C^{14}O_2$ with sucrose and glutamine but it completely inhibited incorporation into asparagine.

TABLE VI

CONCENTRATION, TOTAL ACTIVITY, AND SPECIFIC ACTIVITY OF THE AMIDES AND SUCROSE IN DETACHED TOBACCO LEAVES INFILTRATED WITH EITHER WATER OR SODIUM ARSENITE AND FED $C^{14}O_2$ IN THE LIGHT. PETIOLES WERE IMMERSED IN 0.01 *M* AMMONIUM NITRATE

	Infiltrated with	
	Water	Arsenite
Change in fresh weight, gm.	+2.7	−1.7
Sucrose		
Concentration*	22.10	5.14
Total activity†	6.02	0.90
Spec. activity‡	1.89	1.22
Glutamine		
Concentration*	11.08	5.61
Total activity†	0.39	0.27
Spec. activity‡	0.59	0.81
Asparagine		
Concentration*	11.35	0.0
Total activity†	0.61	0.0
Spec. activity‡	1.35	0.0

*μM. *per gm. fr. wt.*
†μc. *per gm. fr. wt.*
‡μc. *per mgm. C.*

Discussion

While the present data for the synthesis of glutamic acid in broad bean leaves are not consistent with the operation of the usual tricarboxylic acid cycle, they are most easily explained by a suggestion made by Tomlinson (16). He assumed that a series of reactions similar to the tricarboxylic acid occurred, but that the double bond produced in the aconitase reaction was formed between the central carbon atom of *cis*aconitic acid and the carbon atom originating from the methyl group of acetate, and not from the methylene carbon derived from oxalacetate. This change in the position of the double bond formed in the aconitase reaction would result in no labelling in the C_1 of glutamic acid derived initially from labelled carbon dioxide as compared to the large amount of label that would be expected with the operation of the usual tricarboxylic acid cycle. Also this modification in the aconitase reaction would give rise to label in the C_1 of glutamate derived from the carboxyl of acetate. The small amount of label observed in the C_1 of glutamate produced from $C^{14}O_2$ may be attributed to either a limited occurrence of the usual aconitase reaction or to a slight reversal of the α-ketoglutarate dehydrogenase reaction (7). Other explanations of the observed labelling are possible. A C_3 plus C_2 condensation to yield the precursor of glutamate would be consistent with the data.

That aspartic acid in broad bean leaves was not synthesized from oxalacetate produced in the tricarboxylic acid cycle is apparent from the following considerations. Formation of aspartate from oxalacetate produced in the Wood–Werkman reaction would require label from $C^{14}O_2$ to be preferentially incorporated into the β carboxyl of aspartate. In this study the C_2 plus C_3 of aspartate were found to have the highest concentration of label. Aspartate formed from the operation of the tricarboxylic acid cycle would involve the symmetrical intermediate succinate giving an even distribution of label in C_1 and C_4. The observed labelling in C_1 and C_4 was 9 and 23% respectively. If aspartate is formed from some intermediate of the tricarboxylic acid cycle fed essentially by carbohydrate, then the carboxyl groups of aspartate should be preferentially labelled or with the progress of time aspartate should be evenly labelled. The ratio of $\frac{\text{activity in } C_1 \text{ plus } C_4}{\text{activity in } C_2 \text{ plus } C_3}$ was found to be 0.5 throughout photosynthesis from 12 to 125 min. It is clear that some other compound than carbohydrate is continuously contributing a constant fraction of the carbon chain of aspartate. That this might be a C_2 plus C_2 condensation is suggested by Wang *et al.* (18). That acetate may be related to this C_2 compound is indicated by the fact that there was a small amount of label in aspartic acid when acetate-1-C^{14} was fed and utilized without its prior oxidation to $C^{14}O_2$.

Sprinson (13) introduced glycine-2-C^{14} into rats and later on isolated and degraded aspartic acid. He observed that the alpha and beta carbons of such aspartate were two to five times more radioactive than carboxyl carbons. In connection with the present report one should remember that glycine is one of the earliest compounds to become radioactive when $C^{14}O_2$ is fed to leaves in the light.

Conclusions

1. When $C^{14}O_2$ is fed to detached broad bean leaves in light the observed distribution of label in aspartic and glutamic acids precludes the possibility of their formation from the α-keto acids produced by the conventional tricarboxylic acid cycle. Possible mechanisms for the formation of these two amino acids in broad bean leaves are given.

2. The distribution of label in alanine supports the conclusion that this amino acid is formed from pyruvate.

3. It is concluded that aspartic and glutamic acids are the immediate precursors of their corresponding amides.

4. When acetate-1-C^{14} is fed to detached broad bean leaves in the light or dark the only radioactive compounds that can be detected in the alcohol soluble fractions are aspartic acid, glutamic acid, and glutamine. The distribution of label in glutamic acid supports the conclusion that this amino acid is not formed from α-ketoglutarate produced by the conventional tricarboxylic acid cycle.

5. In detached tobacco leaves the formation of asparagine but not that of glutamine is inhibited by arsenite.

References

1. BAKER, N., FEINBERG, H., and HILL, R. Analytical procedures using a combined combustion-diffusion vessel. Simple wet combustion method suitable for routine carbon-14 analyses. Anal. Chem. 26 : 1504-1506. 1954.
2. BIDWELL, R. G. S., KROTKOV, G., and REED, G. B. Synthesis of radioactive glutamine from $C^{14}O_2$ in Swiss chard leaves and its isolation by paper chromatography. Arch. Biochem. and Biophys. 48 : 72-83. 1954.
3. CALVIN, M. and MASSINI, P. The path of carbon in photosynthesis. XX. The steady state. Experientia, 8 : 445-484. 1952.
4. CONNELL, G. E., DIXON, G. H., and HANES, C. S. Quantitative chromatographic methods for the study of enzymatic transpeptidation reactions. Can. J. Biochem. Physiol. 33 : 416-427. 1955.
5. ELLIOTT, W. H. Studies on the enzymic synthesis of glutamine. Biochem. J. 49 : 106-112. 1951.
6. GIBBS, M. Effect of light intensity on the distribution of C^{14} in sunflower leaf metabolites during photosynthesis. Arch. Biochem. and Biophys. 45 : 156-160. 1953.
7. GOLDBERG, M. and SANADI, D. R. Incorporation of labelled carbon dioxide into pyruvate and α-ketoglutarate. J. Am. Chem. Soc. 74 : 4972-4973. 1952.
8. KURSANOV, A. L. A description of the method of vacuum infiltration for the quantitative estimation of the synthesizing and hydrolyzing activity of invertase. Biokhimiyia, 1 : 292-294. 1936.
9. MEISS, A. N. The formation of asparagine in etiolated seedlings of *Lupinus albus* L. Connecticut Agr. Exptl. Sta. Bull. 553. 1952.
10. MEISTER, A. and FRASER, P. E. Enzymatic formation of L-asparagine by transamination. J. Biol. Chem. 210 : 37-43. 1954.
11. MORRIS, D. L. Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Science, 107 : 254-255. 1948.
12. NELSON, C. D. and KROTKOV, G. Specific activities of carbon counted in a methane flow proportional counter either as organic carbon or as barium carbonate. Arch. Biochem. and Biophys. 59 : 294-295. 1955.
13. SPRINSON, D. B. The utilization of the α-carbon atom of glycine for the formation of acetic and aspartic acids. J. Biol. Chem. 178 : 529-530. 1949.
14. STEWARD, F. C. and THOMPSON, J. F. Properties and physiological role of asparagine and glutamine with a new interpretation on the structure of asparagine. Nature, 169 : 739-742. 1952.
15. THOMPSON, J. F. and STEWARD, F. C. Investigations of nitrogen compounds and nitrogen metabolism in plants. II. Variables in two-directional paper chromatography of nitrogen compounds: a quantitative procedure. Plant Physiol. 26 : 421-440. 1951.
16. TOMLINSON, N. Carbon dioxide and acetate utilization by *Clostridium kluyveri*. III. A new path of glutamic acid synthesis. J. Biol. Chem. 209 : 605-609. 1954.
17. VICKERY, H. B., PUCHER, G. W., WAKEMAN, A. J., and LEAVENWORTH, C. S. Chemical investigations of the tobacco plant. VI. Changes that occur in leaves during culture in light and in darkness. Connecticut Agr. Exptl. Sta. Bull. 399. 1937.
18. WANG, C. H., THOMAS, R. C., CHELDENIN, V. H., and CHRISTENSEN, B. E. Conversion of acetate and pyruvate to aspartic acid in yeast. J. Biol. Chem. 197 : 663-667. 1952.
19. WEBSTER, G. C. and VARNER, J. E. Aspartate metabolism and asparagine synthesis in plant systems. J. Biol. Chem. 215 : 91-99. 1955.
20. YEMM, E. W. Glutamine in the metabolism of barley plants. New Phytologist, 48: 315-331. 1949.

STUDIES ON RUBUS VIRUS DISEASES IN BRITISH COLUMBIA

III. SEPARATION OF COMPONENTS OF RASPBERRY MOSAIC[1]

By Richard Stace-Smith[2]

Abstract

It has been shown that the mosaic disease of red raspberry is the result of multiple infection by two separate viruses. Since these viruses, rubus yellow-net and black raspberry necrosis, have a common vector, the aphid *Amphorophora rubi* Kalt., and are both nonpersistent, they are usually transmitted together and the complex nature of the mosaic disease can best be demonstrated by synthesis.

Introduction

This paper is the third of a series reporting investigation of *Rubus* virus diseases in British Columbia. The first (11) reported a disease belonging to the vein chlorosis group. The second (12) reported a disease that was either latent or mild on red raspberry but induced severe symptoms on black raspberry. This paper is concerned with a disease that is characterized on red raspberry varieties by yellowing of the tissue adjacent to the veins, puckering of the interveinal tissue, and down-curling of the leaf blade. It appears to be identical with the severe mosaic disease described from other parts of North America and veinbanding disease in Scotland. In this report the disease will be referred to as raspberry mosaic.

In British Columbia the main raspberry growing district is in the lower Fraser Valley and several fields in this area were surveyed to determine the prevalence of raspberry mosaic. Mosaic was prevalent in most fields of the Newburgh, Cuthbert, Latham, and Lloyd George varieties. The varieties Washington and Willamette were free from mosaic.

Literature

Raspberry mosaic disease is widely distributed throughout the raspberry growing regions of the world. Most of the research work on this disease has been conducted in North America and Great Britain. Since work in these two areas has been independent and the nomenclature adopted for comparable diseases is somewhat confused, the literature is more meaningful if reviewed separately. An attempt will be made in this paper to correlate the North American and European nomenclature.

In North America

In 1902, Stewart and Eustace (13) described a disease of red raspberry in New York that they designated "yellows". Affected plants had yellow,

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mottled, and curled foliage and were generally unproductive. During the next 20 years, a similar disease was recognized in other states and in eastern Canada. Diseases of this type in other crops were termed "mosaics", and in 1922, Rankin and Hockey (10) proposed the name "raspberry mosaic" for this disease. They noted that the most constant and diagnostic symptom of plants affected by mosaic is evident in the spring on the leaves of the suckers, which show large irregular green blisters that arch upward. The tissue between the blisters is lighter green than normal and has a yellowish appearance, and severely blistered leaves curl downward.

In 1924, Wilcox and Smith (14) reported the transmission of mosaic from red to black raspberry by the aphid *Amphorophora rubi* Kalt. Tips of the inoculated black raspberry canes wilted and usually died back one to three inches from the terminal bud. The mosaic on red raspberry varieties that causes cane tip necrosis on black raspberry varieties was named "red raspberry mosaic" by Bennett (1) in 1927. Two other mosaic diseases on black raspberry were named "mild mosaic" and "yellow mosaic". In the following year, Bennett (2) noted that all three mosaics were found on red raspberry.

Further research on raspberry virus diseases demonstrated that classifying all raspberry mosaics into three groups, yellow, mild, and red raspberry mosaic, was unsatisfactory. The various workers agreed that yellow mosaic was a distinct disease of single virus origin. They did not agree, however, on the identity of mild mosaic and red raspberry mosaic, with the result that considerable confusion exists in the nomenclature. In 1931, Rankin (9) used the name "red mosaic" as a substitute for Bennett's red raspberry mosaic and revised the definition to include mild mosaic symptoms. He considered the mild mosaic symptoms and the necrotic symptoms on black raspberry to be phases of the same disease. In 1932, Bennett (3) included mild mosaic symptoms in his description of red raspberry mosaic and he considered that several strains of a virus were responsible for the range in symptom severity. He also suspected that more than one virus was involved in red varieties showing distinct mottling accompanied by green elevations and yellow depressions. Cooley (6) considered that all mosaics other than yellow mosaic were caused by one virus. He suggested the name "green mottle mosaic" as a substitute for red mosaic and red raspberry mosaic.

Recently, Stace-Smith (11, 12) reported experiments on two raspberry virus diseases in British Columbia. One showed a netlike chlorosis of the tissue bordering the smaller leaf veins of four *Rubus* species and the name "rubus yellow-net" was proposed for the disease and causal virus. This disease was similar to yellow mosaic as described by Bennett (3). The other disease exhibited a mild mottling on some red raspberry varieties and was symptomless on other varieties; the causal virus, when inoculated into black raspberry, caused severe stem tip necrosis. The disease was identified with a portion of the red raspberry mosaic group and the name "black raspberry necrosis" was proposed for the disease and virus.

In Great Britain

In 1933, Harris (7) summarized field observations on mosaic diseases of red raspberry in Great Britain and classified them into three main symptom types: *a*, *b*, and *c*. Type *a* symptom, on the variety Mitchell's Seedling, was an evenly distributed chlorotic mottling of the leaves. Type *b* symptoms on the variety Baumforth's B were light and dark green areas of mottling accompanied by a downcurling of the leaflets about the midribs. Type *c* symptoms on Baumforth's B were individual chlorotic, sharply angular, deeply sunken spots on the foliage.

In 1940, Harris (8) reported graft experiments and classified the diseases into two groups, mosaic 1 and mosaic 2. Mosaic 1 showed type *b* symptoms on Baumforth's B, had comparatively limited varietal distribution, and was considered to be of single virus origin. Mosaic 2 comprised two or more diseases of differing intensity: (*a*) mild mosaic 2 with slight and evanescent type *c* leaf symptoms, and (*b*) severe mosaic 2 with intense type *c* leaf symptoms. Harris noted a striking parallel between mosaic 1 and yellow mosaic and between mosaic 2 and red raspberry mosaic as described in America.

In 1951, Cadman (5) reported transmission of a disease of red raspberry in Scotland that he considered to be identical with mosaic 1. He considered that the symptoms were caused by only one virus and he proposed the name "veinbanding" for the disease and causal virus. Cadman graft transmitted the disease to a number of North American varieties and noted that the symptoms were analogous to those of the American red raspberry mosaic.

Materials and Methods

Transmission experiments were undertaken in which apterae of the raspberry aphid, *Amphorophora rubi* Kalt., were used as vectors, and seedlings of black raspberry, *Rubus occidentalis* L., as indicators. The method of aphid manipulation has been discussed in a previous paper (11). Cuthbert, Newburgh, Seedling G, and Latham plants, naturally infected with mosaic, were used as sources of inoculum.

Experimental Procedure

Transmission to Black Raspberry

Colonies of *A. rubi* were allowed to feed for one day on leaves from Cuthbert, Newburgh, and Seedling G plants that showed typical mosaic symptoms. They were then transferred to black raspberry seedlings, 15 aphids from each virus source being placed on three plants, five aphids per plant. Aphids were allowed to feed for six hours on the first indicators, then transferred to the next in the series and permitted to feed for one day, two days, and three days respectively before being destroyed.

Within one week of inoculation, symptoms of virus infection were evident on some of the inoculated plants. The stem terminals became downcurled and brittle and the terminal leaves wilted and died. The tip of the cane

died back two or three inches and the symptoms, in general, were indistinguishable from those caused by black raspberry necrosis virus. Within 10 days after inoculation, all plants of the first, or six-hour, transfer showed black raspberry necrosis symptoms, whereas plants of the second, third, and fourth transfers remained healthy. Plants were held in the greenhouse for two months and virus symptoms did not appear on any of the plants of the last three transfer series. The results of this experiment are summarized in Table I.

TABLE I

INCIDENCE OF BLACK RASPBERRY NECROSIS* ON BLACK RASPBERRY SEEDLINGS INOCULATED BY PROGRESSIVE TRANSFERS OF APHIDS, FIVE PER PLANT, AFTER A FIVE-HOUR ACQUISITION FEEDING ON DIFFERENT SOURCES OF RASPBERRY MOSAIC

Virus source	Duration of successive transfer feedings			
	6 hr.	1 day	2 days	3 days
Cuthbert	3/3	0/3	0/3	0/3
Newburgh	3/3	0/3	0/3	0/3
Seedling G	3/3	0/3	0/3	0/3

**Numerators indicate the number of transmissions; denominators indicate the number of plants inoculated.*

The results of this experiment could be interpreted in one of at least three ways, namely, that: (*a*) mosaic disease is caused by one virus which is transmitted by *A. rubi* and causes symptoms on black raspberry seedlings that are indistinguishable from those caused by black raspberry necrosis virus, (*b*) black raspberry necrosis virus was present in the plants affected by mosaic and this virus unaccompanied by others was transmitted to the test plants, or (*c*) black raspberry necrosis virus plus one or more additional viruses were transmitted to the black raspberry seedlings but the severe effects of the black raspberry necrosis virus masked the presence of the others.

Further aphid transmission experiments were undertaken in an attempt to determine which of the possible explanations represented the true nature of mosaic disease.

Separation of Components

A colony of aphids was transferred from a stock culture in the greenhouse to young leaves detached from a mosaic-diseased Cuthbert plant. After five hours on the inoculum source, 16 aphids were transferred individually through a series of seven black raspberry seedlings. Each aphid was given a transfer feeding of five minutes on the first plant of the series, 10 min. on the second, 15 min. on the third, 30 min. on the fourth, and one hour each on the fifth, sixth, and seventh plants.

Black raspberry necrosis symptoms appeared within one week of inoculation and, within 10 days of inoculation, 28 of the 112 inoculated plants showed symptoms. Twenty days after inoculation, another type of symptom was

noted on one inoculated plant. Small diffuse flecks appeared on one expanded leaf about six inches below the stem tip. Two days later, chlorosis was evident along the veins of the leaf immediately above the one with the initial symptoms. Varying degrees of diffuse netlike chlorosis developed in rapid succession along the veins of the remaining young leaves. The symptoms were identical with those caused by rubus yellow-net virus as previously described on black raspberry seedlings (11). Within 27 days of inoculation, two other plants began to show the same symptoms and, as symptoms developed, these indicators were diagnosed as infected with rubus yellow-net virus. Table II summarizes the separation of two component viruses from the mosaic-diseased Cuthbert plant.

TABLE II

SERIAL TRANSMISSION OF BLACK RASPBERRY NECROSIS (N) AND RUBUS YELLOW-NET (Y) TO BLACK RASPBERRY SEEDLINGS BY SINGLE RASPBERRY APHIDS IN TIMED TRANSFER FEEDINGS, AFTER A FIVE-HOUR ACQUISITION FEEDING ON A MOSAIC-DISEASED RED RASPBERRY

Test aphid number	Duration of successive transfer feedings						
	5 min.	10 min.	15 min.	30 min.	1 hr.	1 hr.	1 hr.
1	N	—	N	—	—	—	—
2	—	N	N	—	—	—	—
3	N	N	N	—	—	—	—
4	—	—	N	N	N	—	—
5	N	—	—	N	—	—	—
6	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—
8	—	N	—	—	—	—	—
9	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—
11	N	Y	N	N	Y	—	—
12	—	N	N	—	—	—	—
13	—	N	N	N	N	N	—
14	—	Y	N	N	—	—	—
15	N	—	—	—	—	—	—
16	—	—	—	N	N	—	—
Total (N)	5	5	8	6	3	1	0
Total (Y)	0	2	0	0	1	0	0

Separation from Other Sources

Further aphid transmission experiments were done to determine whether the two virus components separated from the mosaic-diseased Cuthbert plant could be separated from other sources of mosaic. Two Newburgh, one Latham, one Seedling G, and another selection of Cuthbert, all showing typical mosaic symptoms, were tested. Aphids were given an acquisition feeding of five hours and then placed individually on black raspberry seedlings for a three-hour transfer feeding. Fifty aphids were transferred from the Cuthbert, Seedling G, and one of the Newburgh plants, and 25 aphids were transferred from the Latham and other Newburgh.

Two component viruses, black raspberry necrosis and rubus yellow-net, were separated from each of the sources of mosaic-diseased red raspberry plants. Of the 200 plants inoculated, 82 developed symptoms of black raspberry necrosis within 10 days, and within four weeks 24 plants developed rubus yellow-net symptoms. The results of these experiments are summarized in Table III.

TABLE III

INCIDENCE OF BLACK RASPBERRY NECROSIS AND RUBUS YELLOW-NET ON BLACK RASPBERRY SEEDLINGS FOLLOWING A THREE-HOUR EXPOSURE TO SINGLE APHIDS THAT WERE GIVEN A FIVE-HOUR ACQUISITION FEEDING ON DIFFERENT SOURCES OF RASPBERRY MOSAIC

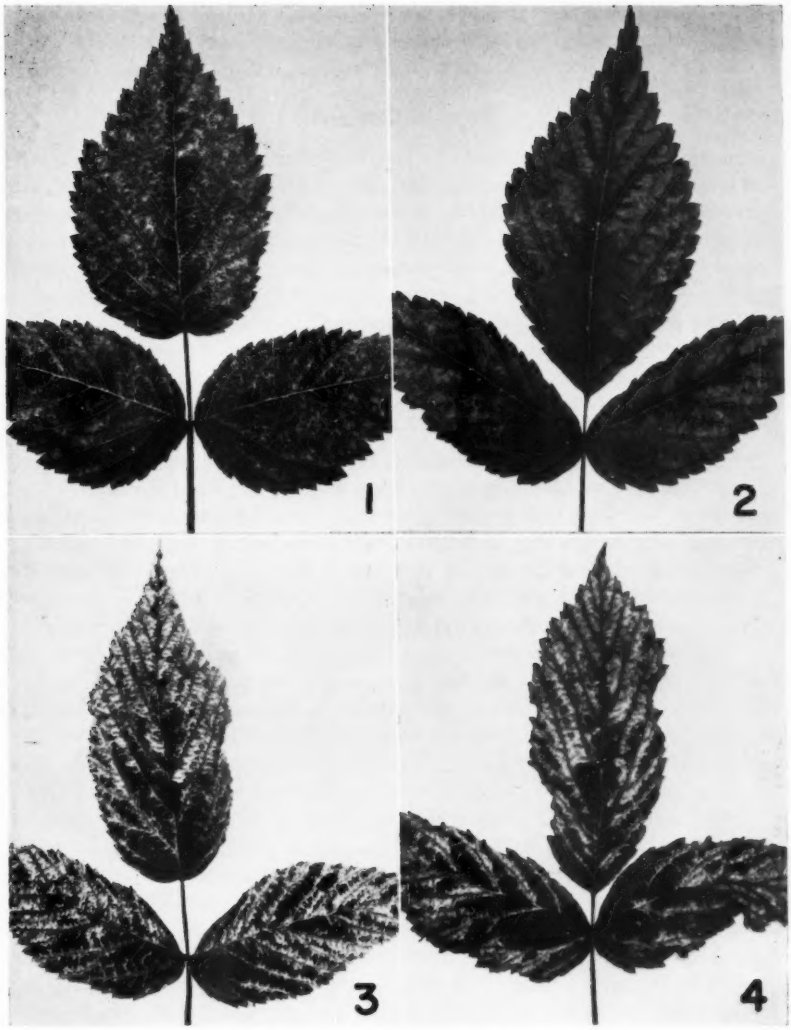
Variety	Number inoculated	Symptom expression	
		Black raspberry necrosis	Rubus yellow-net
Newburgh	50	15	9
Newburgh	25	9	7
Latham	25	13	1
Seedling G	50	24	2
Cuthbert	50	21	5
Total	200	82	24
Percentage of total		41	12

Synthesis of Mosaic Disease

An experiment was undertaken to determine whether typical mosaic symptoms could be induced on red raspberry by combining black raspberry necrosis virus and rubus yellow-net virus, or whether additional viruses were involved in the production of mosaic. Two different stock plants were used: Cuthbert plants that were indexed and proved to be infected with black raspberry necrosis virus, and Washington plants that were indexed and proved to be virus-free. Two Cuthbert plants were grafted with scions from a black raspberry plant infected with rubus yellow-net virus. Two Washington plants were simultaneously grafted with scions from a black raspberry plant infected with rubus yellow-net virus and scions from a Cuthbert plant infected with black raspberry necrosis virus. As a comparison, rubus yellow-net virus alone was grafted to two virus-free Washington plants and two virus-free Cuthbert plants.

In the spring following inoculation, the Washington and Cuthbert plants infected with rubus yellow-net virus alone showed a pale netlike chlorosis of the tissue along the veins. Leaves produced during the summer showed the netlike chlorosis, were slightly cupped downward, and were not distorted (Figs. 1 and 2).

Netlike chlorosis of the tissue along the small leaf veins was evident on the Washington and Cuthbert plants infected with both viruses in the spring following inoculation. Symptoms were more severe than those caused by rubus yellow-net virus alone but not as severe as those on mosaic-diseased



FIGS. 1 and 2. Leaf from a Washington (FIG. 1) and a Cuthbert (FIG. 2) plant showing symptoms of infection with Rubus yellow-net virus.

FIGS. 3 and 4. Leaf from a Washington (FIG. 3) and a Cuthbert (FIG. 4) plant showing symptoms of infection with a mixture of Rubus yellow-net virus and black raspberry necrosis virus.

plants. However, by early summer, yellowing of the tissue adjacent to the leaf veins was extensive, interveinal tissue was puckered, and the leaf blade was downcurled and slightly distorted (Figs. 3 and 4). The symptoms were identical with typical mosaic symptoms observed on naturally infected plants under field conditions.

Discussion

The common names raspberry yellows, raspberry mosaic, red raspberry mosaic, red mosaic, green mottle mosaic, mosaic 1, and veinbanding have been introduced into the literature for a disease that appears to be analogous with the mosaic disease that is prevalent in British Columbia. These names, however, cannot be considered synonymous since some were broad in scope and included a wide range of virus symptoms and others were restricted to the typical mosaic symptoms. The nomenclature is further confused because some of the names were applied to both the disease and the causal virus, which appears to be an erroneous implication. In this paper, the name "raspberry mosaic" is applied to the disease, with the understanding that it is caused by a mixture of two distinct viruses.

Despite environmental and varietal differences, descriptions of raspberry mosaic symptoms from the various raspberry-growing regions of the United States and Canada are relatively uniform, suggesting that a mixture of viruses comparable to those found in British Columbia are responsible for the production of raspberry mosaic symptoms in the different regions. The experiments reported in this paper demonstrate the composite nature of raspberry mosaic, and this finding agrees in principle with Bennett's (3) suspicion that more than one virus is present in mosaic-diseased plants.

The symptoms of raspberry mosaic in British Columbia appear to be analogous with the symptoms of veinbanding as described by Cadman (5) in Scotland, although comparative data on virus composition are lacking. One of the components in British Columbia, black raspberry necrosis virus, can be equated with Cadman's (4) leaf mottle virus. A virus comparable to the rubus yellow-net component, however, has not been described from Europe.

Black raspberry necrosis virus and rubus yellow-net virus are readily transmitted by the aphid *A. rubi*, and the vector relationships of these two viruses do not differ appreciably. As a consequence, when aphids are transferred from a mosaic-diseased plant to black raspberry seedlings, both viruses are usually simultaneously introduced into the indicator, especially when several aphids are transferred to each test plant. When both viruses are introduced simultaneously, the presence of rubus yellow-net virus is not detected because black raspberry necrosis virus destroys the young leaf tissue before the three- to four-week incubation period of rubus yellow-net virus is completed. Thus in the experiment summarized in Table I, when five aphids were transferred to each indicator, rubus yellow-net virus was not separated from black raspberry necrosis virus. However, when indicator plants are exposed to single viruliferous aphids the possibility of both viruses being

introduced simultaneously is reduced. As is shown in the experiments summarized in Tables II and III, rubus yellow-net virus is occasionally transmitted unaccompanied by black raspberry necrosis virus in single-aphid transmission experiments.

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References

1. Bennett, C. W. Virus diseases of raspberries. Mich. Agr. Expt. Sta. Tech. Bull. 80. 1927.
2. Bennett, C. W. Michigan raspberry diseases. Mich. Agr. Expt. Sta. Spec. Bull. 178. 1928.
3. Bennett, C. W. Further observations and experiments with mosaic diseases of raspberries, blackberries, and dewberries. Mich. Agr. Expt. Sta. Tech. Bull. 125. 1932.
4. Cadman, C. H. Studies in *Rubus* virus diseases. I. A latent virus of Norfolk Giant raspberry. Ann. Appl. Biol. 38 : 801-811. 1951.
5. Cadman, C. H. Studies in *Rubus* virus diseases. III. A veinbanding disease of raspberries. Ann. Appl. Biol. 39 : 69-77. 1952.
6. Cooley, L. M. The identity of raspberry mosaics. Phytopathology, 26 : 44-56. 1936.
7. Harris, R. V. Mosaic disease of the raspberry in Great Britain. I. Symptoms and varietal susceptibility. J. Pomol. Hort. Sci. 11 : 237-255. 1933.
8. Harris, R. V. Mosaic disease of the raspberry in Great Britain. II. Experiments in transmission and symptom analysis. J. Pomol. Hort. Sci. 17 : 318-343. 1940.
9. Rankin, W. H. Virus diseases of black raspberries. New York State Agr. Expt. Sta. Tech. Bull. 175. 1931.
10. Rankin, W. H. and Hockey, J. F. Mosaic and leaf curl (yellows) of the cultivated red raspberry. Phytopathology, 12 : 253-264. 1922.
11. Stace-Smith, R. Studies on *Rubus* virus diseases in British Columbia. I. Rubus yellow-net. Can. J. Botany, 33 : 269-274. 1955.
12. Stace-Smith, R. Studies on *Rubus* virus diseases in British Columbia. II. Black raspberry necrosis. Can. J. Botany, 33 : 314-322. 1955.
13. Stewart, F. C. and Eustace, H. J. Raspberry cane blight and raspberry yellows. New York State Agr. Expt. Sta. Bull. 226. 1902.
14. Wilcox, R. B. and Smith, F. F. Transfer of mosaic disease from red to black raspberries. Phytopathology (Abstr.), 14 : 55. 1924.

COMPOSITION OF TURPENTINE OF LODGEPOLE × JACK PINE HYBRIDS[1]

By N. T. Mirov

Abstract

Chemical composition of turpentines in lodgepole-jack pine hybrids was studied. When the two pines were artificially crossed, the F_1 generation possessed turpentine in which the bicyclic terpenes of jack pine dominated over the simpler, monocyclic terpene—phellandrene—of lodgepole pine. Trees in a natural hybrid swarm also tended towards a predominance of the jack pine bicyclic terpenes.

Very little is known about the inheritance of chemical characters in plants. If one parent "*A*" has a chemical compound "*a*" and another parent "*B*" has instead a compound "*b*" of the same class of compounds (e.g. terpenes, organic acids, or any other class), what kind of compound may be expected to be present in the hybrid between "*A*" and "*B*" ? What kind of compound would be found in a hybrid swarm, when the "*A*" plant and the "*B*" plant meet and cross under natural conditions ?

This paper attempts to shed some light on these two questions. The plants under consideration are lodgepole pine (*Pinus contorta* Dougl.) and jack pine (*Pinus banksiana* Lamb.). The investigated compounds belong to the class of unsaturated predominantly cyclic compounds—terpenes.

Range and Taxonomy of Lodgepole and Jack Pines

Both lodgepole pine and jack pine belong to the group *Insignes* of the subgenus *Diploxylon* genus *Pinus* (25). Lodgepole pine has a wide range, being found from lower California to Alaska and Yukon territory, and from the sea level of the Pacific to an elevation of 11,000 ft. in the Rocky Mountains.

Lodgepole pine was first collected in 1826 by Mertens near Sitka, Alaska, during his voyage as a botanist and zoologist of Captain Lütke's around-the-world expedition (15). The specimens were studied by Bongard (4) and the pine was described as *Pinus inops* Ait., which is a synonym for *Pinus virginiana* Mill. Comparing his material with the drawing of *P. inops* in Lambert's *Description of genus Pinus* (11), Bongard, apparently not sure of the identity of his Alaskan pine, made a note that "spinae squamarum parum breviores, quam in iconae Lamberti laudata."

Some botanists distinguish coastal and inland forms of lodgepole pine. The Pacific coast form is sometimes designated as *Pinus contorta* Dougl., whereas the inland form, both of the Sierra Nevada of California and the

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American Rockies, is known as *P. contorta* var. *latifolia* Engelm., as *P. contorta* var. *murrayana* (Grev. & Balf.) Engelm., or as *Pinus murrayana* Grev. & Balf. A scrubby form of the pine found on the Pacific coast of California is called *P. contorta* var. *bolanderi* Parl.

In British Columbia the coastal form and the inland form merge, and farther north both go under the name of *P. contorta*. In Alberta, lodgepole pine descends somewhat from the eastern slope of the Rockies to the adjacent plains.

Jack pine, or Banks pine, is named after Sir Joseph Banks, once President of the Royal Society of London. It is a northeastern pine occupying an enormous range from Nova Scotia and Maine westward to Minnesota, Ontario, Manitoba, Saskatchewan, northeastern Alberta, northeastern British Columbia, and the Mackenzie and the Yukon regions. Halliday and Brown (8) state that jack pine and lodgepole pine "meet along the eastern foothills of the Rocky Mountains overlapping in the Peace river districts." More accurately, in Alberta, in an area located between Edmonton on the south, Peace River on the north, Grande Prairie on the west, and a point not far from Athabaska on the east, there are numerous groves where the two pines are intermingled. Here one may observe trees possessing morphological characters intermediate between the characters of lodgepole pine and jack pine. These intermediate forms in the "overlap" area were thoroughly studied in 1947 and 1948 by Moss (18), who arrived at the conclusion that they are hybrids between the two species.

In 1939, Righter (22) succeeded in crossing lodgepole pine and jack pine. Pollen was collected from a jack pine in the Eddy Arboretum, Institute of Forest Genetics, Placerville, California, grown from seed obtained from Michigan. The seed parent was a wild lodgepole pine growing at an elevation of 5700 ft. in El Dorado County, California. The resulting hybrid possessed morphological characters intermediate between the parents.

Morphological differences between lodgepole pine, jack pine, and an artificial F_1 hybrid are largely of a comparative nature. Lodgepole pine cones are spreading or reflexed, each scale carrying a strong recurved spine. Jack pine cones are predominantly erect, recurved towards the apex of the shoot—seldom spreading, but always unarmed. The hybrid cones generally resemble those of lodgepole pine, but armed with slender inconspicuous prickles.

Ecological Aspects of Natural Hybridization

In the overlap area lodgepole pine grows on the heavier soils and is associated with white spruce (*Picea glauca* (Moench) Voss), quaking aspen (*Populus tremuloides* Michx.), and balsam poplar (*Populus balsamifera* L.). Jack pine occupies poorer sites on sandy areas where it grows together with scrubby quaking aspen and paper birch (*Betula papyrifera* Marsh.). The patches of forest trees in the overlap area alternate with stretches of grassland and cultivated fields.

During the Wisconsin glaciation, the jack pine forests of Canada were completely destroyed. When forest trees reinvaded Canada, jack pine came as a component of the eastern forest centered on the Appalachian land mass, that is, it approached Alberta from the east. Lodgepole pine migrated southward, probably from the Yukon Valley (9), even before the Wisconsin glaciation and may have persisted in ice-free areas close to the ice front during at least the late Wisconsin glaciation when the land located both eastward and westward was still covered with ice.

In early postglacial times what is today the overlap area probably was an extensive grassland, or perhaps a parkland or grove area. The black soils now found frequently in the area could not have developed under postglacial conifer forests. Moss (19) concluded that the forest is gradually encroaching upon the grassland. Fires and cultivation have retarded this encroachment. All investigators agree that the area has been greatly disturbed by fires, which became more frequent with the advent of primitive man. Although Moss (19) considers the disturbing influence of bison and domestic animals only of local importance, nevertheless, judging by the information available for other grassland regions (3), the influence of once-numerous hoofed wild animals in disturbing the prehistoric grasslands should not be disregarded. We may visualize the Alberta overlap area since the retreat of the ice as having been repeatedly disturbed by fires, by wild cattle, and by possible subsequent surface erosion especially in sandy places. The area offered conditions favorable to the appearance of lodgepole × jack pine hybrid swarm (1). It might be possible that the two pines never overlapped, but merely approached each other when hybrids were first formed. The hybrid swarm then might have originated in a disturbed area not previously occupied by either parent.

There is no botanical evidence at present of a preglacial hybridization between the two pines. Apparently there had been no preglacial contact between lodgepole pine and jack pine; at least no fossil records are available that would indicate such contact. No signs of hybridization between these two pines have been reported in any part of their respective ranges other than the Alberta overlap area.

A slight variability in the cone morphology of jack pine was mentioned by Moss (19, p. 228). Moss suggested that the horizontal cones of jack pine occasionally found as far east as Manitoba might be the result of a "postglacial union of lodgepole and typical jack pine" although he was cautious to add that the occurrence "seems to make a considerable demand upon a migration hypothesis." In later correspondence Dr. Moss informed the author that jack pine possessing horizontal cones has been found as far east as northern Michigan. In the author's opinion, the straight-cone jack pine may be considered as a variant within the species, developed without any introgression of lodgepole pine genes. Racial variability of jack pine throughout its range, described by Schantz-Hansen and Jensen (23), is strictly infraspecific; there also is no evidence of introgression of lodgepole pine genes. At the Institute of Forest Genetics, Placerville, California, there is a jack pine from Connecticut whose otherwise typical cones are horizontal, with the tips curved down.

Pines of an intermediate character are found occasionally west of and beyond the overlap area, as for instance near Seebe, between Calgary and Banff. There are also reports of intermediate forms in Saskatchewan. Both of these localities are not too far from the overlap area of Alberta.

Chemical Composition of Turpentines

When the trunk of a pine is cut, the wound yields a quantity of oleoresin. The oleoresin consists of a part that is volatile with steam, called turpentine, and of a non-volatile part, called rosin. Commonly turpentine is composed of terpenes. Terpenes offer certain advantages over other plant constituents in a study of inheritance of chemical characters: terpenes are an end product of metabolism (12) and once formed, they are apparently not used by the plant; turpentine composition in all but a few pine species is very simple; and enough material can be obtained from an individual tree for chemical analysis.

The chemical composition of turpentine is specific. It may vary considerably within a species, but the variability is quantitative rather than qualitative in character. The chemical variability is reflected in a variability of physical characters of pine turpentine: its density, index of refraction, and optical rotation. Variability of the rotatory power, for instance, may be caused by varying amounts of different terpenes or by the presence of levo- and dextro-antipodes of the same terpene in varying amounts. A pure dextrorotatory antipode of alpha-pinene rotates the plane of polarized light to about $+40°$. The rotation of a mixture of the two antipodes may vary all the way from $+40°$ to $-40°$. Again, the variability of optical rotation of a turpentine may be caused by presence of two different terpenes, one dextro- and the other levorotatory, as in jack pine.

Optical rotation of jack pine turpentine fluctuates considerably. This fluctuation is caused both by varying amounts of levo-β-pinene and by varying amounts of dextrorotatory and levorotatory antipodes of α-pinene. Some samples of jack pine turpentine from Ontario and from Wisconsin are levorotatory. All jack pine turpentine samples from Alberta were dextrorotatory.

Lodgepole pine turpentine from all localities, except Sierra Nevada mountains of California, is levorotatory. It consists almost entirely of levo-β-phellandrene. The Sierra Nevada lodgepole turpentine contains about 10% of a dextrorotatory sesquiterpene-ketone. This semisolid compound apparently is not found in the Canadian lodgepole pine in appreciable quantity. Thus, jack pine turpentine consists of a mixture of dextro- and levo-α-pinene, with an admixture of levo-β-pinene, both terpenes being of bicyclic structure. No phellandrene is present (7, 16). Canadian lodgepole turpentine consists almost entirely of a monocyclic terpene, levo-β-phellandrene (24). The structure of α-pinene, β-pinene, and β-phellandrene is shown in Fig. 1. There are thus clear chemical differences between the turpentines of lodgepole and jack pines. What, then, is the nature of the turpentine in the hybrid between these two pines ?

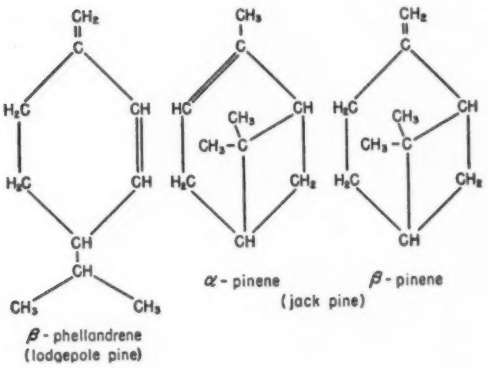

FIG. 1. Structural formulae of the terpenes of lodgepole and jack pines. Note that phellandrene has conjugated double bonds (p. 451).

Source of Experimental Material

Several samples of turpentine from typical lodgepole pine from California, Idaho, and Colorado were analyzed by the writer before the present study. Samples analyzed for this study were from Kananaskis Forest Experiment Station near Calgary, Alberta; from Kamloops, British Columbia; and from trees planted in New Zealand. Turpentine samples of typical jack pines were obtained from Minnesota, Wisconsin, and Ontario.[2]

Samples of turpentine from the artificial lodgepole pine × jack pine hybrid were obtained at the Institute of Forest Genetics, Placerville, California, where the hybrid was developed (22). Six F_1 trees, 12 years old, about 6 in. in diameter at the base, and 20 ft. tall, were tapped in 1952; a small quantity of oleoresin was obtained, not sufficient to analyze the sample individually but enough for a composite sample. The turpentine was dextrorotatory ($\alpha_{578\ m\mu} = +7.00°$), had an index of refraction, $n_D^{25} = 1.4713$, and a density, $d_4^{25} = 0.8520$. Fractional distillation and identification of the compounds revealed the presence of 75 to 78% pinenes and 20 to 22% phellandrene. The conclusion was that the F_1 generation of an artificially produced (and thus authentic) hybrid between lodgepole pine and jack pine possessed turpentine of a mixed nature.

Oleoresin of 73 individual trees intermediate between lodgepole and jack pines was obtained from the overlap area near Edmonton, Alberta, where these two pines meet and apparently cross (Fig. 2). In the summer of 1952 the writer visited the Edmonton area and with the kind assistance of Dr. Moss collected oleoresin samples from two localities, one northeast of Edmonton,

[2]*Thanks are due to the members of the Kananaskis Forest Experiment Station, to the members of the Provincial Forest Service of British Columbia, to the Forest Officers of Ontario, and to the Lake States Forest Experiment Station, U.S. Forest Service, for their assistance in procuring the oleoresin samples.*

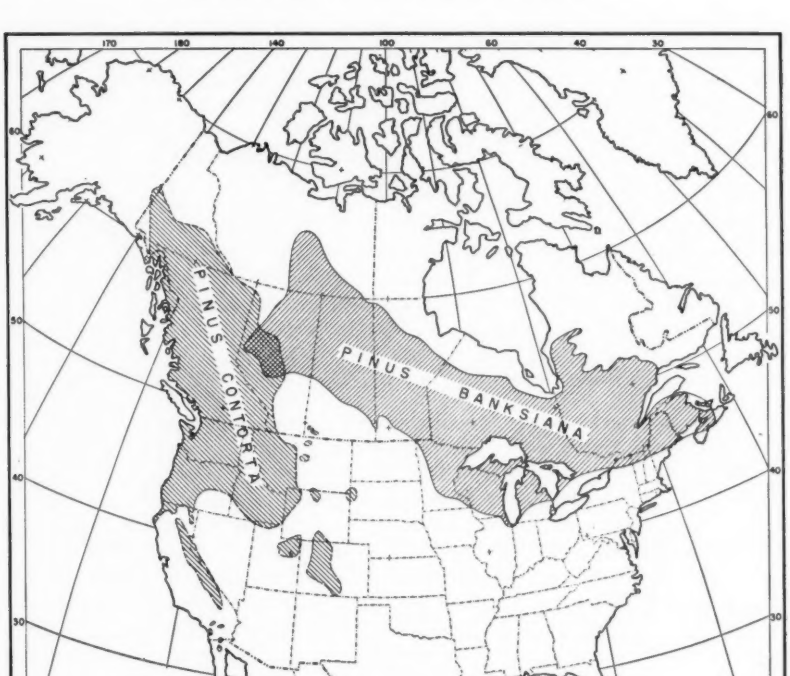

FIG. 2. Geographic range of jack pine (*P. banksiana*) and lodgepole pine (*P. contorta*) showing the Alberta overlap area.

near Bruderheim, designated in this paper as Station M, and another northwest of Edmonton, designated as Station N. In the summer of 1954, oleoresin samples from 10 other localities between Edmonton and the Rockies were collected under the supervision of Dr. Moss (Fig. 3). All these samples furnished experimental material used in the present study (Table I).

Experimental Technique

Oleoresin samples were collected from the pines by cutting V-shaped grooves ½ in. deep into the sapwood. The exuded oleoresin was collected in glass jars and kept in cold storage until used.

The turpentine was obtained by heating oleoresin *in vacuo*, so that, at the end of the operation, when all turpentine was distilled off, the pot temperature was increased to 180° C. and pressure was reduced to 1 mm. of mercury. The distilled turpentine samples then were dried over anhydrous sodium sulphate

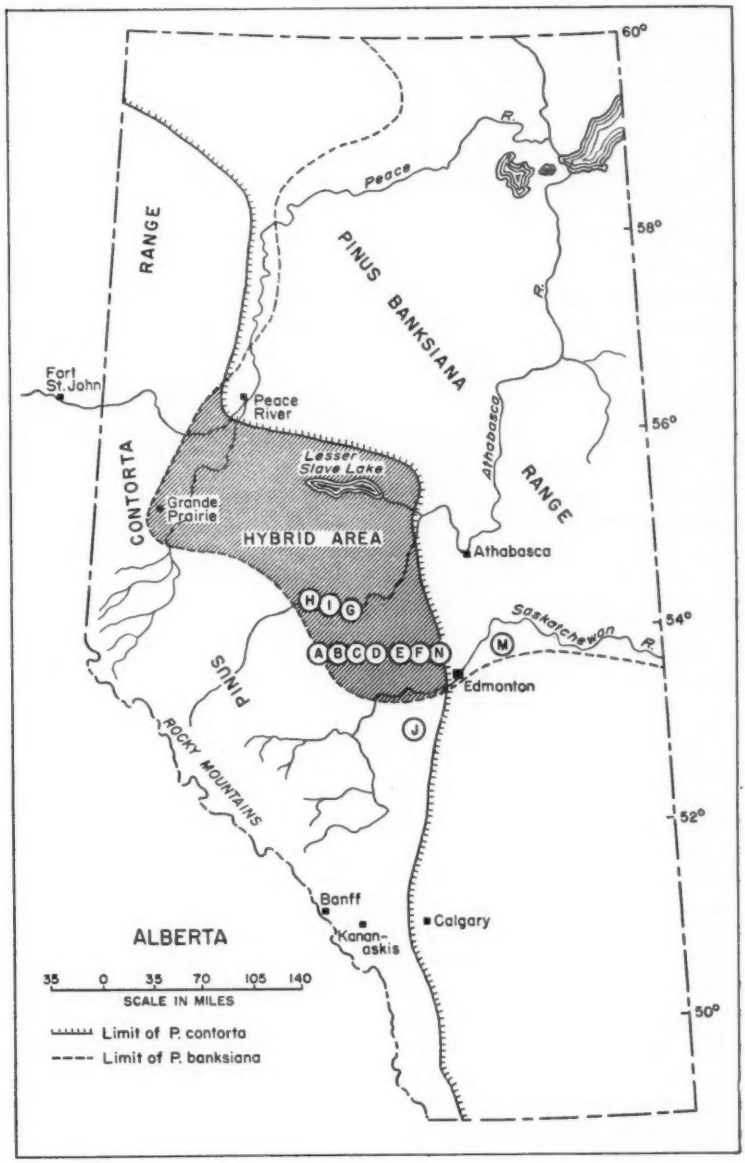

FIG. 3. The Alberta overlap area, showing the location of experimental plots.

TABLE I

SOURCE OF TURPENTINE SAMPLES

Location	Plot symbol	No. trees in plot	No. trees from which turpentine samples were obtained*	Classification of trees on morphological characters†
Lodgepole—jack pine overlap area				
Bruderheim, 30 mi. NE. of Edmonton	M	11	9	Typical jack pine
Heatherdown, 35 mi. NW. of Edmonton	N	10	9	Lodgepole, hybrids, and typical jack pine
21 mi. SW. of Edson (140 mi. W. of Edmonton)	A	11	10	Typical lodgepole
19 mi. SW. of Edson	B	7	5	Typical lodgepole
10 mi. SW. of Edson (130 miles W. of Edmonton)	C	9	7	Typical lodgepole
Near Leaman, 80 mi. W. of Edmonton	D	11	7	All but one tree typical lodgepole
Near Evansburg, 60 mi. W. of Edmonton	E	10	1	Mixture of hybrids and typical lodgepole
Near Seebe, 50 mi. W. of Edmonton	F	10	1	All typical jack pine
95 mi. NW. of Edmonton (4 miles E. of White Court)	G	10	8	Jack pine and hybrids
125 mi. NW. of Edmonton (30 miles W. of White Court)	H	6	3	Typical lodgepole
15 mi. W. of White Court (110 miles NW. of Edmonton)	I	8	3	Lodgepole and hybrids
Near Rocky Mt. House, about 100 miles SW. of Edmonton	J	12	10	Typical lodgepole with an admixture of possible hybrids
Total for hybrid area		115	73	
Other areas				
Artificial hybrid *P. contorta* × *P. banksiana* (Institute of Forest Genetics, Calif.)		1 composed sample		Cones with weak spines
P. contorta (including *P. murrayana*) from California, British Columbia, and Alberta		12 composed samples		Cones with strong spines
P. banksiana from Minnesota, Wisconsin, and Ontario		4 composed samples		Cones smooth (without spines)

**Not all trees yielded enough oleoresin for chemical analysis.*
†Based on description of Dr. Moss and on herbarium specimens.

and kept in a refrigerator. Altogether, 90 turpentine samples were prepared and investigated: 4 samples from jack pine, 12 samples from lodgepole pine, 1 sample from the artificial hybrid between these two pines, and 73 samples from the individual trees of the Alberta hybrid area. For each sample the following characters were determined: density, d_4^{25}; index of refraction, n_D^{25}; specific rotation, $[\alpha]_{578m\mu}^{23}$.

Density of the turpentine, d_4^t was determined by means of a pycnometer of 0.4 cc. capacity. A factor of 0.0008 per degree was used to correct the reading to 25° C. Index of refraction n_D^t was determined by means of Abbe

refractometer and corrected to 25° C. by using a factor 0.00045 per degree.[3] Optical rotation was observed with a polarimeter, using a standard 1-decimeter long tube. The specific rotation was calculated by dividing the observed optical rotation of the sample by its density. The rotation measurements were made in the mercury yellow, using Corning glass filters Nos. 3480 and 4303. The resultant wavelength was 578 mμ.

Phellandrene (in its levo-β-form) was identified by preparation of phellandrene nitrosite, according to the method suggested by Baker and Smith (2); α-pinene was identified by its nitrosochloride (28).

Thirty-nine samples of turpentine were subjected to an ultraviolet ray analysis.[4] Presence of conjugated double bonds in the lodgepole pine phellandrene and absence of conjugation in the jack pine turpentine permitted an easy differentiation between the turpentines of the two pines by this method.

Pure β-phellandrene has an absorption maximum at 232 mμ in the ultraviolet and the ϵ value is 19,300 (5). This ϵ value was used to calculate the percentage of phellandrene by use of Beer's law. Since the validity of Beer's law (13) for solutions of phellandrene in 95% ethanol has not been checked, the percentages of phellandrene calculated are to be regarded not as highly accurate measurements but as indices of the relative amount present.

The transmission values were measured at 232 mμ for solutions of the turpentine in 95% ethanol, mostly at a dilution of 10^{-4} molar. A dilution of 10^{-3} or 10^{-2} molar was used for samples having a low absorption value. The values were determined on a Beckman DU model spectrophotometer.

Results

The indices of refraction and the densities of the 73 individual trees of the overlap area were plotted by a method adopted from the work of Sutherland (27). This diagram (Fig. 4) shows that the chemical composition of turpentine of the individual trees was far from uniform. Some points for individual trees are concentrated along the α-pinene–β-pinene line where points for pure jack pine samples from the eastern part of the continent are located. On the lower right end of the diagram are located pines possessing lodgepole pine characteristics. The values for true lodgepole pines from outside the hybrid swarm are scattered much more than the values for the jack pines. This scattering is probably caused mostly by the varying sesquiterpene fraction of the lodgepole pine turpentine, but the position of the two composed samples from Kananaskis, Alberta (between Calgary and Banff), may possibly be caused by the presence of pinenes from jack pine. Between these two extremes of the diagram are scattered the individual trees that possess turpentines intermediate between that of lodgepole and jack pine. Turpentine from the artificial F_1 hybrid also had characteristics like those of the intermediate group.

[3]*Correction of readings to 25° C. was necessary for preparation of Sutherland diagram (Fig. 4).*

[4]*Thanks are due to Dr. P. M. Iloff, Jr., for furnishing the spectrophotometric data for the turpentine samples.*

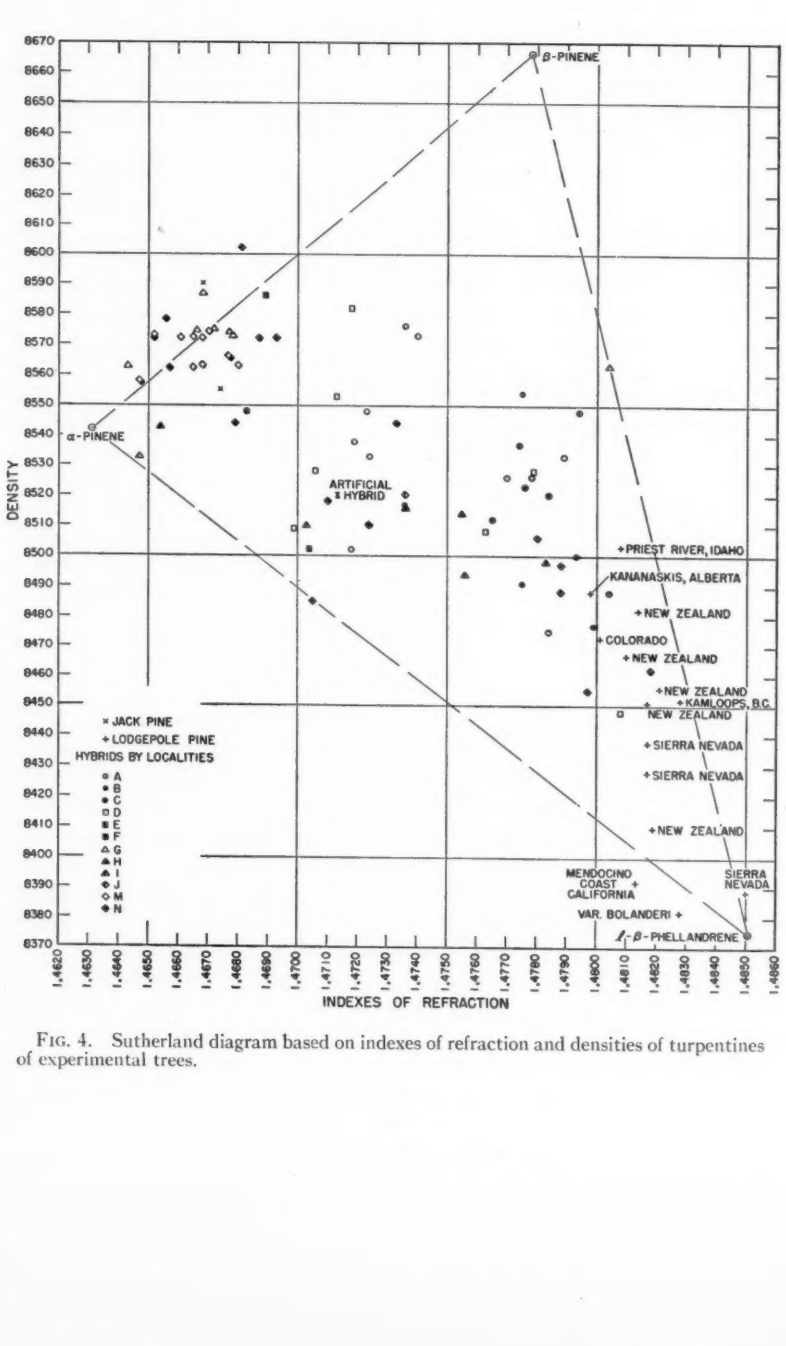

FIG. 4. Sutherland diagram based on indexes of refraction and densities of turpentines of experimental trees.

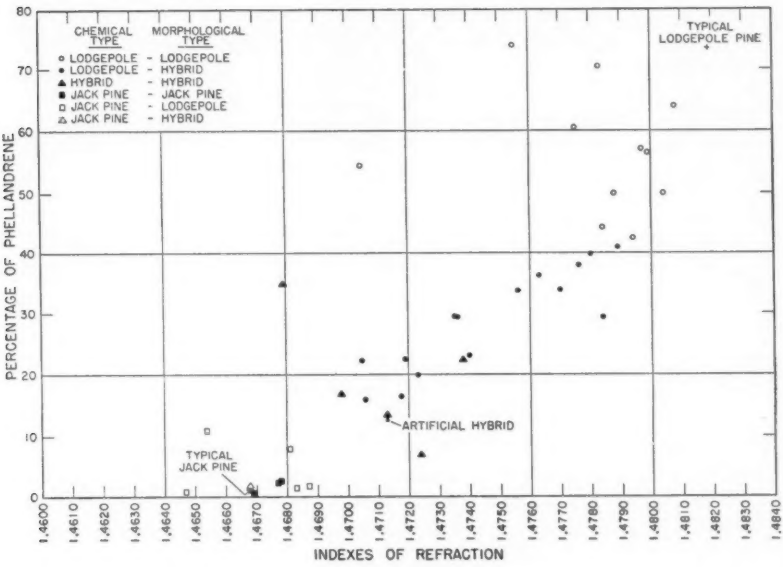

FIG. 5. Phellandrene content of experimental trees and the indexes of refraction.

Results of photospectrometric determination of phellandrene (Fig. 5) suggest that turpentine obtained from 39 individual trees in the Alberta overlap area had rather low 1-β-phellandrene content; even the pure lodgepole pine turpentine contained only 73%. This relatively low figure may be explained by inaccuracies of spectroscopic determination and possibly by polymerization of phellandrene, which is a very unstable terpene; nevertheless, the position of the pure lodgepole pine turpentine on the diagram may serve as a criterion for estimating the relative content of phellandrene in the individual trees of the hybrid swarm. Of these, the presumably pinene (that is, jack) pines are clustered at the lower left end of the diagram. At the upper right part are located apparently pure phellandrene (that is, lodgepole) pines. These are much more scattered than the pinene pines. The artificial hybrid is located closer to the pinene pines and probably actually contains 20 to 22% of phellandrene, judging from the previously mentioned fractional distillation.

Specific rotation was plotted (Fig. 6) with the trees arranged in the same order as they appeared on the Sutherland diagram; that is trees possessing dextrorotatory turpentine, characteristic of jack pine, to the left and trees possessing levorotatory turpentine, characteristic of lodgepole pine, to the right. It is apparent from this diagram that the trees in the overlap area are to a larger extent dextrorotatory, possessing the physical character of jack pine turpentine. Data on morphological and chemical characters are presented in Table II.

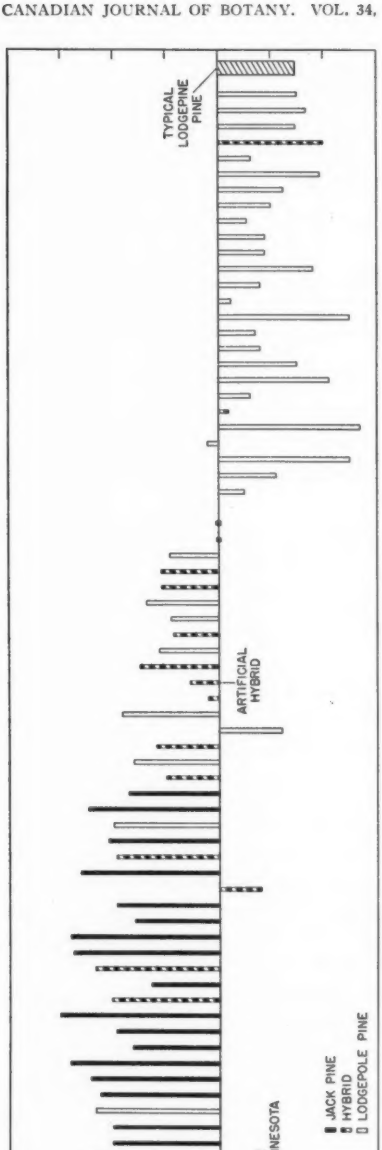

FIG. 6. Specific rotation of turpentines of experimental trees as they appear from left to right on Fig. 4.

TABLE II

DISTRIBUTION OF EXPERIMENTAL TREES FROM THE ALBERTA LODGEPOLE–JACK PINE OVERLAP AREA, BY MORPHOLOGICAL AND CHEMICAL CHARACTERS

Morphological character	Chemically jack pine		Chemically intermediate forms		Chemically lodgepole pine		Total	
	No.	%	No.	%	No.	%	No.	%
Jack pine	21	28.8	0	0	0	0	21	28.8
Intermediate	3	4.1	14	19.2	0	0	17	23.3
Lodgepole pine	3	4.1	17	23.3	15	20.5	35	47.9
All	27	37.0	31	42.5	15	20.5	73	100.0

Summary and Discussion

Results of our findings may be summarized as follows:

1. Lodgepole pine turpentine consisted largely of levo-β-phellandrene with varying amounts of dextrorotatory sesquiterpene compounds. (Sesquiterpenes amounted to a considerable percentage in trees of the Sierra Nevada of California and almost none in trees of the coast and the Rocky Mountains.)

2. Jack pine turpentine consisted predominantly of a mixture of dextro- and levo-α-pinene (more dextro-antipode in Alberta than in Ontario and Lake States), with a small and varying admixture of levo-β-pinene.

3. The artificial hybrid between lodgepole pine and jack pine possessed turpentine consisting of a mixture of levo-β-phellandrene (20 to 22%) and pinenes (75 to 78%).

4. The pines sampled in Alberta where ranges of the two species overlap were of a heterogeneous morphological appearance. Some looked like jack pine; others appeared to be of an intermediate type; trees that resembled lodgepole pine were most numerous.

5. Chemical composition of turpentines of the overlap area pines did not always correspond to morphological features of the pines.

From analysis of the morphological and chemical characters of the 73 experimental trees from the overlap area (Table II), the following conclusions can be drawn:

1. None of the pines that could be easily identified morphologically in the field as jack pines possessed the lodgepole compound, phellandrene. Nearly 30% of the 73 trees were of this type.

2. Pines that externally appeared to be lodgepole pines were found to be either phellandrene pines, that is, chemically lodgepole (20% of the total); or chemical hybrids, possessing both jack pine pinenes and lodgepole pine phellandrene in different proportions (23%); or chemically pure jack pines (4%).

3. Pines that possessed morphological characters intermediate between jack pine and lodgepole pine were also often chemically intermediate (about 20%

of the total). Of the morphologically intermediate pines 4% were chemically pure jack pines; none in this morphologically intermediate group was chemically pure lodgepole.

The present investigations of inheritance of terpenes in lodgepole–jack pine hybrids show that when the two pines were artifically crossed, the F_1 generation possessed turpentine in which the bicyclic terpenes of jack pine dominated over the simpler monocyclic terpene—phellandrene—of lodgepole pine. The ratio was about 3 to 1. Trees in the natural hybrid swarm around Edmonton, Alberta, also tended toward a predominance of the jack pine pinenes. Morphologically, however, more trees in the hybrid swarm looked like lodgepole pines than like jack pines.

Dominance of complicated turpentine components over simpler ones has been previously found in turpentine of the hybrid between *Pinus ponderosa* (containing a mixture of bicyclic terpenes) and *Pinus jeffreyi* (containing a saturated straight chain hydrocarbon of the methane series, *n*-heptane) (14).[5] This trend also seems apparent in the natural cross of *Pinus montezumae* and *Pinus pseudostrobus* encountered by the author in Chiapas, Mexico (10, 17).

A similar phenomenon has been observed in a study of inheritance of essential oil components in *Ocimum* by Nilov (20). There are indications, although not expressed, of the same nature in the works of Penfold and his associates (21) with Australian Myrtaceae and in Sievers' experiments with hybridization of species of the genus *Mentha* (26).

The present study is admittedly of a preliminary character. It was concerned with only one group of chemical substances found in plants—terpenes. To study other classes of organic compounds seems highly advisable. If a gradual disappearance of simpler compounds in natural or in artificial hybrids is of widespread occurrence in nature, this phenomenon might be important in the study of evolution of chemical substances in plants.

References

1. Anderson, E. Introgressive hybridization. John Wiley & Sons, Inc., New York. 1949.
2. Baker, R. T. and Smith, H. G. A research on the Eucalypts, especially in regard to their essential oils. Technol. Museum, New South Wales. Tech. Educ. Ser. No. 13. 1902. p. 262.
3. Berg, L. S. Natural regions of the U.S.S.R. The MacMillan Co., New York. 1950. p. 105.
4. Bongard, H. G. Observations sur la végétation de l'île de Sitcha. Mem. Imp. Acad. Sci. St. Petersburg, 6th Ser. No. 143. 2 : 163. 1833.
5. Davenport, J. B., Sutherland, M. D., and West, T. F. 'β-phellandrene' from Canada Balsam oil. J. Appl. Chem. 1 : 527-528. 1951.
6. Guenther, E. The essential oils. Vol. 2. D. Van Nostrand Company, Inc., New York. 1949.
7. Haagen-Smit, A. J., Redemann, C. T., Wang, T. H., and Mirov, N. T. Composition of gum turpentines of pines. A report on *P. ponderosa*, *P. banksiana*, *P. canariensis* and *P. washoensis*. J. Am. Pharm. Assoc. Sci. Ed. 39 : 260-265. 1950.[6]

[5]*The U.S. Forest Products Laboratory Report on which this information was based gives a ratio of 25% heptane and 75% terpenes.*

[6]*In the last column of Table III of this article the first two figures should have a plus sign instead of the minus. The α-pinene in P. banksiana is predominantly dextrorotatory.*

8. HALLIDAY, W. E. D. and BROWN, A. W. A. The distribution of some important forest trees in Canada. Ecology, 24 : 353-373. 1943.
9. HANSEN, H. P. Postglacial forests in West Central Alberta, Canada. Bull. Torrey Botan. Club, 76 : 278. 1949.
10. ILOFF, P. M. and MIROV, N. T. Composition of gum turpentines of pines XVII. A report on *P. montezumae* from Chiapas and *P. oocarpa* var. *trifoliata* and *P. durangensis* from Durango, Mexico. J. Am. Pharm. Assoc. Sci. Ed. 42 : 46-49. 1953.
11. LAMBERT, A. B. A description of the genus *Pinus*, etc. 2nd ed. Vol. 1. Weddell, London. 1828.
12. LVOV, S. D. Signification physiologique du process de la formation des essences pour la plante. Acad. Sci. Bot. Soc. U.S.S.R. Essays Botan. Moscow, 2 : 561-676. 1954.
13. MELLON, M. G. Analytical absorption spectroscopy. John Wiley & Sons, Inc., New York. 1950.
14. MIROV, N. T. A note on Jeffrey and Western Yellow pines. J. Forestry, 30 : 93-94. 1932.
15. MIROV, N. T. Lodgepole pine discovered and misnamed. Madroño, 12 : 156-157. 1954.
16. MIROV, N. T. Chemical composition of gum turpentines of pines of the United States and Canada. J. Forest Products Research Soc. 4 : 107. 1954.
17. MIROV, N. T. Composition of turpentines of Mexican pines. Unasylva, 8 : 167-173. 1954.
18. MOSS, E. H. Natural pine hybrids in Alberta. Can. J. Research, C, 27 : 218-229. 1949.
19. MOSS, E. H. Grassland of the Peace River region, Western Canada. Can. J. Botany, 30 : 98-124. 1952.
20. NILOV, V. I. Chemical changes in plant hybridization. All-Union Acad. Agr. Sci. Nikitskii Botan. Garden, 171-192. 1939.
21. PENFOLD, A. R. Researches on essential oils of the Australian flora. Museum Technol. Appl. Sci. Sydney, 1948-1953.
22. RIGHTER, F. I. and STOCKWELL, W. P. The fertile species hybrid *Pinus murraybanksiana*. Madroño, 10 : 65-69. 1949.
23. SCHANTZ-HANSEN, T. and JENSEN, R. A. The effect of source of seed on growth of jack pine. J. Forestry, 50 : 539-544. 1952.
24. SCHORGER, A. W. Contribution to the chemistry of American conifers. Wisc. Acad. Sci. Trans. Pt. 2. 1919.
25. SHAW, G. R. The genus *Pinus*. Publs. Arnold Arboretum No. 5, Cambridge, Mass. 1914.
26. SIEVERS, A. F., LOWMAN, M. S., and RUTTLE, M. L. Investigations of the yield and quantity of the oils from some hybrid and tetraploid mints. J. Am. Pharm. Assoc. Sci. Ed. 34 : 225-231. 1945.
27. SUTHERLAND, M. D. A review of densities and refractive indices of terpenes. Univ. Queensland Papers, Dept. Chem. 1 : 1-21. 1948.
28. WALLACH, O. Zur Kenntniss der terpenen. Ann. 245 : 253. 1888.

EFFECT OF NUTRITION ON GROWTH AND CHLAMYDOSPORE FORMATION IN BROWN AND GRAY CULTURES OF THIELAVIOPSIS BASICOLA[1]

By R. H. Stover[2]

Abstract

Physiological differences between brown and gray wild type cultures of *Thielaviopsis basicola* were sought in order to develop selective media for use in mutational studies, and as a basis for pathogenicity and ecological studies. The gray wild type was more thiamine-deficient, yielded less mycelium, and grew more slowly than the brown on all media tested regardless of composition and thiamine content. In liquid media both types were more thiamine-deficient on sucrose than on glucose, but the reverse was true on agar media. The gray type was inhibited more than the brown by malachite and methyl green. A two-layered thiamine-deficient agar medium, and one containing dyes, was selective for the brown type. Gray and brown types grew better on thiamine-deficient water agar and soil extract agar than on thiamine-deficient agar media containing 20 gm./l. of sugar and 1 gm./l. of nitrogen compounds. On thiamine-deficient media, distortion and extrusion of the contents of endoconidia and hyphae were more common with the gray wild type than the brown.

Endoconidia, but not chlamydospores, were formed in varying amounts on all thiamine-deficient media. The addition of thiamine resulted in the abundant production of chlamydospores and greatly increased growth. Chlamydospore production was also influenced by the source of nitrogen. Certain amino acids were more inhibitory to chlamydospore formation by the brown than gray type and vice versa. Asparagine, for example, blocked chlamydospore formation by the brown but not the gray type, even though growth and endoconidial production were abundant. Mutants capable of overcoming this blockage were readily produced in culture.

Introduction

Thielaviopsis basicola (Berk. & Br.) Ferraris exists in nature in two distinct racial groups designated brown and gray wild types (7, 8, 9) which are easily distinguished on potato dextrose agar by their pigmentation. For mutational, pathogenicity, and ecological studies, it would be advantageous to have a method of readily separating mixed cultures of the two types. Therefore studies were begun to determine some of the physiological differences between the brown and gray types that might be utilized in developing selective media.

Literature Review

Lilly and Barnett (4) included *T. basicola* among 57 specimens of fungi grown on 12 sugars for various incubation periods with asparagine monohydrate as a source of nitrogen. They found that *T. basicola* utilized *d*-glucose, *d*-fructose, *d*-mannose, *d*-galactose, maltose, sucrose, and cellobiose

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rapidly, *l*-arabinose and raffinose slowly, and *l*-sorbose, *d*-xylose, and lactose slightly or not at all. *d*-Glucose yielded more mycelium more rapidly than sucrose for the first 4 days but at 9 and 13 days sucrose outyielded glucose. Fructose was more slowly utilized than glucose but at 13 days the yields were approximately the same. Autoclaving glucose compared to filtering reduced the yield of *T. basicola* slightly. Autoclaved fructose yielded only one-half or less that of filtered fructose. Of the component sugars of raffinose, *T. basicola* utilized melibiose slightly and fructose rapidly. When these two sugars were present together the yield was greater than on fructose alone indicating that melibiose is utilized in the presence of fructose. The presence of sorbose with glucose or maltose inhibited the utilization of the two latter sugars by *T. basicola*.

Trezzi *et al.* (10) determined the effect of a large number of carbon and nitrogen sources including amino acids on the growth of *T. basicola*.

Lilly and Barnett (3) list *T. basicola* among the thiamine-deficient fungi requiring an exogenous source of thiamine. Neither the pyrimidine nor the thiazole moiety was utilized separately or combined by the strain of the fungus studied (2). However, allithiamine yielded about one-half the growth of thiamine (5). Steinberg (6) showed that *T. basicola* required potassium, phosphorus, magnesium, sulphur, and the micronutrients iron, zinc, copper, manganese, and molybdenum. The addition of calcium to an asparagine–sucrose medium caused substantial increases in yield.

Methods and Materials

A brown-type culture of *T. basicola* was obtained from a cotton stem from New Mexico and a gray-type from tobacco roots from Blacksburg, Virginia.* Although the usual precautions (7) were taken to prevent displacement and eventual loss of the wild types by cultural mutants, these developed in some instances and were the most important cause of variation in results.

Each liter of basal medium was made up of reagent-grade K_2HPO_4, 1 gm.; KCl, 0.5 gm.; $MgSO_4$, 0.5 gm.; and $FeSO_4$, 0.01 gm.; thiamine hydrochloride, 100 or 500 μgm. Asparagine monohydrate or $(NH_4)_2SO_4$ at a suboptimal concentration of 1 gm. was the usual nitrogen source and sucrose or glucose at 20 gm. was the carbon source. Amino acid content was calculated on the basis of nitrogen equivalent to that contained in 1 gm. of asparagine. No adjustments for pH were made. With $(NH_4)_2SO_4$ and glucose, pH varied from 6.2 to 6.8 and with sucrose, from 6.8 to 7.4. The pH was slightly higher with asparagine than with $(NH_4)_2SO_4$.

When thiamine requirements were being studied, media were treated with Norit (animal charcoal) at 5 gm. per liter (3). Glassware was washed in concentrated sulphuric acid and rinsed five times in tap water and six times in distilled water. Extreme care was required to prevent introduction of thiamine into the culture flasks with the inoculum. Dry aerial mycelium and

**Drs. P. J. Leyendecker and R. G. Henderson kindly provided this diseased plant material.*

spores were removed from an actively growing agar colony with a fine needle, care being taken not to touch the agar substrate, and introduced directly into the culture flasks.

Agar media were prepared by adding 1.7% Difco agar to the liquid media. Where thiamine-deficient agar medium was used, the agar-free solution was treated with Norit, the agar, which probably contained a trace of thiamine (1), was added and the medium sterilized. Soil extract agar was prepared by heating 1000 cc. of soil in 1000 ml. of water for 30 min. in flowing stream in the autoclave. The extract was filtered clear and volume increased to one liter with distilled water. Tobacco root agar was made by adding 2 gm. of fresh young White Mammoth tobacco roots to test tubes containing 10 ml. of water-agar, autoclaving, and slanting. Where Difco dehydrated potato dextrose agar (P.D.A.) was not used, fresh P.D.A. was made from a clear extract of 200 gm. of potatoes heated in distilled water in the autoclave for five minutes at 15 lb. and brought up to 1 liter with distilled water containing 10 gm. of dextrose and 2% agar.

Liquid media were dispensed in 25 ml. lots into 125 ml. flasks and autoclaved at 15 lb. for 15 min. Inoculum was added to the flasks which were incubated for seven or eight days in a drawer at room temperature (24°–32° C.). Five flasks were utilized for each treatment. Mycelium and spores from each flask were collected on tared filter paper in a Buchner funnel, dried to constant weight, weighed, and the yield expressed in milligrams.

Results

I. Growth on Liquid Media

Atypical Growth

In liquid cultures mutants or mutant-like variants resulted in an atypical growth, and were a common source of variation in results. Instead of the usual diffuse mycelial mat and pellicle of spores resulting from wild-type growth, a few large or numerous small mycelial 'balls' appeared. Frequently, only a single ball of mycelium about 2–4 mm. in diameter would form from the inoculum. These cultures yielded much less than characteristic wild types. Atypical growth appeared to be more common with gray-type inoculum and on synthetic media containing inorganic sources of nitrogen. When 10% or more of the flasks showed atypical growth, experiments were repeated with new sources of inoculum. The use of young, freshly monospored cultures for inoculum helped to reduce variants.

Utilization of Different Nitrogen and Carbon Sources

Different sources of nitrogen were tested with sucrose and glucose. An exploratory experiment showed that NO_3 and NO_2 nitrogen were not utilized by brown or gray *T. basicola*. Organic sources of nitrogen were superior to inorganic although all forms of NH_4 nitrogen tested were utilized by the brown and gray types. Of the compounds tested, peptone gave the highest yield with potato extract, asparagine, and $(NH_4)_2SO_4$ following in that order.

In one experiment, NH_4NO_3 was compared with $(NH_4)_2SO_4$, nitrogen being calculated as that equivalent to 1 gm. of $(NH_4)_2SO_4$ or asparagine per liter. Growth with NH_4NO_3 was less than with $(NH_4)_2SO_4$. This was attributed to a nitrogen deficiency resulting from unavailability of NO_3 nitrogen. When the nitrogen equivalent was calculated from the NH_4 radical alone, NH_4NO_3 yielded as much mycelium as $(NH_4)_2SO_4$. In this experiment, growth of both types in glucose was greater than in sucrose but the difference was most pronounced where there was a large nitrogen deficiency resulting from the use of NH_4NO_3. That the quantity of nitrogen utilized was suboptimal, even with $(NH_4)_2SO_4$, is indicated by the response of the fungus to different quantities of asparagine (Table I).

TABLE I

YIELDS OF BROWN *T. basicola* AT DIFFERENT CONCENTRATIONS OF ASPARAGINE IN BASAL MEDIA CONTAINING 20 GM. OF SUGAR PER LITER

	Gm. asparagine per liter				
	0.5	1	1.5	2	2.5
Carbon source	Yield in mgm.				
Sucrose	23	27	43	47	50
Glucose	20	44	57	62	62

T. basicola was more highly pigmented with asparagine as a source of nitrogen than with $(NH_4)_2SO_4$. Also, the medium contained a brown pigment and the higher the asparagine content, up to 2 gm./l., the greater was the pigmentation of the medium.

Figures in Table II show that the gray wild type grew more slowly and yielded less than the brown on the two nitrogen and three carbon sources tested. Asparagine was the better form of nitrogen, and glucose was superior to sucrose where asparagine was utilized. However, there was little difference between glucose and sucrose with $(NH_4)_2SO_4$ nitrogen. In an experiment not shown, maltose and potato starch proved to be good sources of carbon and maltose was superior to sucrose with both $(NH_4)_2SO_4$ and asparagine.

TABLE II

GROWTH OF BROWN AND GRAY CULTURES OF *T. basicola* WITH DIFFERENT SOURCES OF CARBON AND NITROGEN

		Carbon source 20 gm./l.		
Culture	Nitrogen source, 1 gm./l.	Sucrose	Glucose	Levulose
		Yield in mgm.		
Gray	$(NH_4)_2SO_4$	75	73	39
Brown	$(NH_4)_2SO_4$	78	81	54
Gray	Asparagine	82	144	65
Brown	Asparagine	104	179	70

Effects of Thiamine and Thiamine Deficiency on Growth

In the first experiments, gray, brown, and gray and brown mixed cultures were tested on $(NH_4)_2SO_4$–sucrose thiamine-deficient medium. There was no difference in the behavior of gray and brown mixed cultures and brown cultures alone; therefore, the brown cultures were compared with the gray. In Table III, IV, V, these data show that gray-type *T. basicola* is more thiamine-deficient than brown and thiamine deficiency is greater with sucrose than glucose. Also, gray cultures are slower metabolizers and yield less per gram of carbon and nitrogen than the brown.

TABLE III

YIELD IN MGM. OF GRAY AND BROWN CULTURES OF *T. basicola* ON THIAMINE-DEFICIENT MEDIUM WITH $(NH_4)_2SO_4$ NITROGEN

	Cultural type	
Carbon source 20 gm./l.	Gray	Brown
Sucrose	0.1	3.8
Glucose	4	15

TABLE IV

YIELD IN MGM. OF GRAY AND BROWN CULTURES OF *T. basicola* WITH DIFFERENT QUANTITIES OF THIAMINE AND $(NH_4)_2SO_4$ (1 GM./L.) NITROGEN

	μgm./l. of thiamine				
Carbon source 10 gm./l.	0	50	125	250	500
Sucrose					
Gray	3	10	13	13	14
Brown	27	37	42	41	41
Glucose					
Gray	12	22	21	24	22
Brown	23	25	25	28	26

TABLE V

YIELD IN MGM. OF GRAY AND BROWN WILD TYPES OF *T. basicola* WITH DIFFERENT QUANTITIES OF THIAMINE, AND $(NH_4)_2SO_4$ AND ASPARAGINE NITROGEN

	Thiamine, μgm./l.			Thiamine, μgm./l.		
	0	50	250	0	50	250
	Gray			Brown		
Asparagine						
Sucrose	5	30	33	10	38	37
Glucose	17	37	38	20	54	64
$(NH_4)_2SO_4$						
Sucrose	4	36	39	14	44	43
Glucose	18	43	41	28	43	43

Effects of $CaCO_3$ and Buffers on Growth

The pH changes of liquid cultures after eight days were as follows: $(NH_4)_2SO_4$–sucrose medium from 7.0–7.3 to 4.0–6.6; $(NH_4)_2SO_4$–glucose medium from 6.7–6.9 to 6.1–6.6. The addition of $CaCO_3$ (1 gm./l.) to these media lowered the acidity and doubled the yield. When asparagine was the source of nitrogen, however, pH changes were not as pronounced and $CaCO_3$ caused a slight increase in yield with sucrose but not with glucose. With gray cultures the addition of calcium to $(NH_4)_2SO_4$ nitrogen caused a reduction in density of the spore pellicle and increased pigmentation of the mycelium. No attempts were made to determine the effects of calcium as a trace element (6).

The addition of Na_2HPO_4 and NaH_2PO_4 (1 gm./l.) to the basal medium containing $(NH_4)_2SO_4$ increased the yield and the former buffer was superior to the latter. The best yield was obtained from $(NH_4)_2HPO_4$ alone as an inorganic source of nitrogen and a buffer.

II. Growth on Different Agar Media

Agar media cultures were obtained by placing in the center of each plate 0.1 ml. of an aqueous spore suspension containing two spores per microscope field of 100 ×, Table VI, or by a pin-point needle transfer of dry inoculum, Table VII. On agar media with asparagine and $(NH_4)_2SO_4$, sucrose was slightly superior to glucose. In thiamine-deficient agar media without dyes, Table VI, the fungus was more deficient on glucose than on sucrose. The effect of method of inoculation may be noted in Tables VI and VII. Radial growth was reduced where a single spot-inoculation of dry spores was used, Table VII, and was vigorous with drop-inoculation containing a dilute spore suspension in distilled water, Table VI.

TABLE VI

GROWTH OF GRAY AND BROWN CULTURES OF *T. basicola* AFTER SIX DAYS ON AGAR SUBSTRATES, SPOT-INOCULATED WITH AN AQUEOUS SUSPENSION OF SPORES

	Thiamine added, 100 μgm./l.		No thiamine added	
	Growth in mm.	Observations	Growth in mm.	Observations
Asparagine				
Sucrose Brown	50	Good, dense growth and color differentiation	45	4–6 mm. yeast-like colonies in center with sparse mycelial strands radiating out
Gray	34		10	
Glucose Brown	46		25	
Gray	28		8	
$(NH_4)_2SO_4$				
Sucrose Brown	45	Fair growth, poor color differentiation	45	Strands of gray type may radiate outward on one side of yeast-like colony
Gray	29		10	
Glucose Brown	24		24	
Gray	19		4	
Water-agar				
Brown	—		40	Small thin yeast-like colonies in center with sparse mycelial growth radiating out; S.E.A. has less radial growth than water-agar but is 3–4 times more dense
Gray	—		23	
Soil extract agar				
Brown	—		36	
Gray	—		22	

TABLE VII

GROWTH OF GRAY AND BROWN *T. basicola* ON AGAR SUBSTRATES SPOT-INOCULATED WITH DRY SPORES ON A NEEDLE

Carbon–nitrogen source and pH of media	Thiamine added 100 μgm./l.			No thiamine added		
	Growth diam. in mm.		Observations, 6 days	Growth diam. in mm.		Observations, 6 days
	4 days	6 days		4 days	6 days	
Asparagine						
Sucrose Brown	23	38	Good pigmentation and color differentiation dense, raised surface growth, browns and grays easily distinguished	17	22	Yeast-like colonies (2–5 mm.) developed at point of inoculations, thin, submerged rhizoid strands may grow out from these, growth very sparse
7.6 Gray	11	30		0	0*	
Glucose Brown	18	32		0	0*	
7.4 Gray	0	10		0	0	
$(NH_4)_2SO_4$						
Sucrose Brown	17	22	Fair to poor pigmentation and color differentiation, growth appressed	0*	21	Yeast-like colonies (2–5 mm.) developed at point of inoculations, thin, submerged rhizoid strands may grow out from these, growth very sparse
7.3 Gray	7	16		0	0	
Glucose Brown	0	7				
6.7 Gray	0	0				
Water-agar						
6.9 Brown	—	—	—	20	22	Sparsely prod. rhizoid strands grow out radially from small (2–4 mm.) yeast-like colonies
Gray	—	—	—	5	10	
Soil extract agar						
6.8 Brown	—	—	—	14	16	Radial growth less than above but more dense; mycelium: gray type, wavy; brown straight
Gray	—	—	—	5	8	
Old Difco P.D.A.						
+NaOH Brown	—	—	—	30	35	Good radial growth mostly submerged, sparse spore production on surface contrasted with asparagine–sucrose
6.9 Gray	—	—	—	14	15	

**One-third of plates had a strand of mycelium growing out radially from the yeast-like colonies at point of inoculations.*

When brown colonies grew on plates of $(NH_4)_2SO_4$–sucrose and $(NH_4)_2SO_4$–glucose agar, the colonies were antagonistic to each other. With asparagine, however, the colonies grew together and covered the surface of the plate. This may be due partially to pH changes since the pH in the intercolony space of the $(NH_4)_2SO_4$ medium was 5.2 and in the asparagine, 6.6.

The morphology and physiology of brown-type growth on thiamine-deficient agar medium with $(NH_4)_2SO_4$ as a nitrogen source differed from those of the gray. Abortion and extrusion of contents of spores and hyphae were more prevalent with gray cultures than with brown. Also there was a tendency for brown-type hyphae to form round vesicular chlamydospore-like structures which later collapsed.

When gray and brown type spores were dispersed throughout a thiamine-deficient agar substrate, the brown germinated and grew to the surface or sporulated more quickly than did the gray. This suggested a possible method of selecting brown types in a mixed population of brown and gray. Therefore, spores were incorporated into a layer 2–3 mm. thick of warm agar medium which was solidified and then covered with another layer of agar 2–3 mm. thick. After three and four days the surface of the second layer was examined with the low power objective and the number of colonies reaching the surface and sporulating were counted for gray and brown types, Table VIII. Since the brown type was less thiamine-deficient than the gray, it grew more rapidly reaching the substrate surface sooner and sporulating.

TABLE VIII

NUMBER OF COLONIES OF GRAY AND BROWN TYPES OF *T. basicola* SPORULATING ON SURFACE OF ONE AND TWO-LAYERED THIAMINE-DEFICIENT AGAR MEDIA WITH $(NH_4)_2SO_4$ NITROGEN

		% of fields* with surface sporulation			
		Gray		Brown	
Carbon source	Days	I†	II	I	II
Glucose	3	46	0	80	26
	4	62	9	95	55
Sucrose	3	40	0	74	38
	4	60	5	80	50

* *Each figure derived from an examination of 25 fields at random over agar surface on six plates; each plate contained from 125–200 colonies.*

† *I = one layer of media, II = two layers of media.*

Exploratory experiments showed that certain dyes incorporated into Difco P.D.A. were more inhibitory to the gray type than to the brown. Equal quantities of the spores of the two types were placed in Petri plates and Difco P.D.A. containing dyes in various concentrations was added. Developing colonies were removed at random and transferred to tubes of P.D.A. for identification. The data in Table IX show that media containing malachite

TABLE IX

NUMBERS OF GRAY AND BROWN COLONIES OF *T. basicola* FROM A MIXED POPULATION ON AGAR MEDIA WITH AND WITHOUT DYES

	Experiment			
	1		2	
	Gray	Brown	Gray	Brown
Difco potato dextrose (D.P.D.A.)	11	15	74	34
D.P.D.A. + rose bengal 1 : 50,000	10	8	63	28
D.P.D.A. + methyl green 1 : 100,000	0	25	25	95
D.P.D.A. + malachite green 1 : 400,000	0	29	—	—

TABLE X

GROWTH OF GRAY AND BROWN CULTURES OF *T. basicola* AFTER THREE DAYS ON DIFFERENT MEDIA CONTAINING DYES AND THIAMINE

		Nutrient source							
		Glucose				Sucrose			
Dye	Culture	Asparagine		$(NH_4)_2SO_4$		Asparagine		$(NH_4)_2SO_4$	
		1*	2†	1	2	1	2	1	2
1 : 100,000 methyl green	Gray	3	5–15	2	5–15	2	5–15	1	5–15
	Brown	6	50–200	6	25–50	4	50–100	4	25–50
1 : 400,000 malachite green	Gray	1	5–15	0	0–5	1	0–15	0	0–15
	Brown	5	50–200	3	25–50	4	50–100	2	10–15

*1 = *Macroscopic growth, 6 is maximum score.*
†2 = *Microscopic growth, length of germ tubes, 200 is maximum range in microns.*

and methyl green were partially selective for brown type. Also, these dyes reduced the concentration and size of gray colonies but had less effect on the brown. These dyes were also incorporated into $(NH_4)_2SO_4$–sucrose, $(NH_4)_2SO_4$–glucose, asparagine–sucrose, and asparagine-glucose media with and without thiamine. Growth was so poor on thiamine-deficient media with malachite and methyl green that the data were not recorded. Results for the other media are shown in Table X. Growth on glucose media with dyes is better than on sucrose and malachite is more inhibitory than methyl green. However, without these dyes, growth was better on sucrose than on glucose, Table VIII. The gray type was inhibited to a greater extent than the brown on all media tested.

Methyl green was incorporated into media containing various proportions of gray and brown spores distributed at random. After four to five days, developing colonies were removed and transferred to slants of asparagine-sucrose agar for identification, Table XI. The selective action of the dye for the brown type and the greater selectivity with $(NH_4)_2SO_4$ nitrogen

TABLE XI

PROPORTION OF GRAY TO BROWN CULTURES OF *T. basicola* OBTAINED FROM DIFFERENT MEDIA CONTAINING MIXED POPULATIONS OF GRAY AND BROWN TYPES IN DIFFERENT RATIOS

Media	Ratio of gray:brown spores added to media			
	1 : 1, no dye	1 : 1	2 : 1	5 : 1
		1 : 100,000 methyl green		
$(NH_4)_2SO_4$–glucose	66*	1	0	8
	61	64	78	85
Asparagine–sucrose	34	17	2	15
	118	65	71	53
Asparagine–glucose	29	8	12	21
	52	51	54	42
$(NH_4)_2SO_4$–sucrose	28	5	5	4
	56	25	40	47

**Proportion of gray (upper figure) to brown type colonies (lower figure) obtained from media.*

contrasted with asparagine is evident, Table XI. On $(NH_4)_2SO_4$–sucrose medium with methyl green, numerous endoconidia formed into round thin-walled spores several times larger than the original spore. These ball-like structures also formed on the ends of hyphae but were absent on asparagine–sucrose media with methyl green.

III. Chlamydospore Production

Endoconidia but not chlamydospores formed in varying amounts on all media. Data in Table XII show that chlamydospores do not form on thiamine-deficient media or on old-stock dehydrated Difco P.D.A., although growth in the latter is better than in other thiamine-deficient media and water or soil extract agars. In water and soil extract agars, chlamydospores did not appear until after 10 to 14 days and were small (two to four segments) and sparsely produced. They appeared adjacent to thiamine-producing bacterial and fungal contaminants on thiamine-deficient media and certain contaminants and antagonists slightly stimulated production on soil extract agar. Certain antagonists growing in association with *T. basicola* in soil extract agar caused the usually straight hyphal growth of the brown type to become wavy like that of the gray in the vicinity of the antagonist. On soil extract agar, with or without contaminants, gray-type chlamydospores usually formed several days later and were less abundant than the brown.

Data in Tables XIII and XIV show that chlamydospore production by the brown and gray forms of *T. basicola* varies with the nitrogen source and

TABLE XII

CHLAMYDOSPORE PRODUCTION BY *T. basicola* ON DIFFERENT MEDIA

Media	When formed days	Numbers	Number of pigmented segments
Soil extract agar	6–12	Few*	2–4
Water-agar	10–14	Few*	2–4
Liquid media $(NH_4)_2SO_4$ and asparagine, glucose and sucrose with thiamine added	4–6	Abundant	4–6
No thiamine	0	0	0
Agar media with added thiamine and *fresh* P.D.A. without added thiamine	6–8	Abundant	4–6
No thiamine	0	0	0
Old Difco P.D.A. pH 5.6 and old Difco P.D.A. + NaOH pH 6.9	0†	0	0
$(NH_4)_2SO_4$–sucrose with thiamine and 1 : 100,000 methyl green	6–8	Abundant	3–4
Asparagine–glucose with thiamine and 1 : 100,000 methyl green	6–8	Abundant	5–10
Tobacco roots (autoclaved)	5–7	Abundant	5–6

**More common when colony is growing near certain bacterial and fungal contaminants.*

†*A few chlamydospores were produced at point of inoculation and are attributed to the presence of thiamine in the inocula.*

TABLE XIII

CHLAMYDOSPORE PRODUCTION BY BROWN AND GRAY *T. basicola* ON DIFFERENT MEDIA IN TEST TUBES AFTER EIGHT DAYS

	Brown		Gray	
	Carbon source			
N Source	Glucose	Sucrose	Glucose	Sucrose
Methionine	+++++*	++++	+++++	+++++
Cystine	+++++	+++	+++++	+++
Proline	+++++	—	+++++	—
Glutamic acid	+++++	—	+++++	—
$(NH_4)_2SO_4$	+++++	+++++	+++++	+++++
Glycine	++++	++	++++	++++
Leucine-methionine	++++	+++	+++++	+++++
Tryptophane	++++	++	++++	+++
Leucine	+++	0	++++	+++
Cysteine	+++	++++	+++	+++++
Leucine-tryptophane	+++	0	+++	+++
Lysine	++	+	+	+
Tyrosine	++	+	++	+++
Histidine	++	+	++	++
Control	++	—	++	—
Leucine-histidine	+	0	+++	+++
Arginine	0	0	++	+
Leucine-arginine	0	0	+++++	+++

* +++++, *excellent production;* ++++, *good;* +++, *fair;* ++, *poor;* +, *very poor;* 0, *none.*

TABLE XIV

CHLAMYDOSPORE PRODUCTION AFTER 10 DAYS BY BROWN AND GRAY *T. basicola* ON MEDIA CONTAINING GLUCOSE WITH DIFFERENT SOURCES OF NITROGEN

	Substrate					
	Agar in test tubes		Agar in plates		Liquid in flasks	
N Source 1 : 1	Brown	Gray	Brown	Gray	Brown	Gray
Arginine–$(NH_4)_2SO_4$	+++++*	++	0	++	+	+
Arginine–cystine	+++++	+	+	++++	+++++	+++++
Arginine–glycine	++++	+++	+	+++++	+++++	+++++
Arginine–leucine	+	++++	+	++++	+++	+++++

* +++++, *excellent production;* ++++, *good;* +++, *fair;* ++, *poor;* +, *very poor;* 0, *none.*

cultural environments. In general, gray-type chlamydospore formation was less inhibited by certain amino acids than brown type. The brown type did not form chlamydospores when arginine or leucine–arginine were sources of nitrogen but the gray did. After a period of 10 days on agar substrates containing arginine, 'patch' mutants and mycelial overgrowths that contained chlamydospores were observed in brown-type cultures. Thus chlamydospore-free brown colonies on arginine media soon give rise to 'mutants' capable of forming chlamydospores with arginine. Endoconidial production and fungus growth regardless of the number of chlamydospores produced were abundant on most of the media containing amino acids.

Discussion

Few qualitative nutritional differences were found that would easily distinguish the gray from the brown type in early stages of colony development. The gray type differed quantitatively from the brown, however, in a slower growth rate and usually lower yields. The slower growth rate of the gray type may be related to a lower level of carbohydrate metabolism than the brown as suggested by greater thiamine deficiency and sensitivity to dyes. Also, there were important differences between types in chlamydospore formation with certain amino acids, such as the blockage of chlamydospore formation in the brown type by arginine. In general, the sulphur-bearing amino acids stimulated chlamydospore formation. Amino acids may be useful in developing a medium where a large number of colonies can be quickly distinguished as to type by scanning culture plates with a microscope for the absence or presence of chlamydospores.

From these and other studies (7, 8, 9), gray *T. basicola* differs most significantly from the brown as follows: (1) less pathogenic, (2) slower growing, (3) more thiamine-deficient, (4) survives shorter periods under dormancy, (5) is less inhibited by certain amino nitrogen sources in formation of chlamydospores. The first four characteristics indicate the gray is less likely to survive than the brown in mixed populations. However, the gray type has a wider nutritional spectrum with regard to chlamydospore formation which would

increase its adaptability to hosts. Nevertheless, the over-all evidence indicates that the brown type is more advanced parasitically than the gray and can attack more vigorously a wider range of hosts (8, 9). Since the gray type exists, at least in localized areas, in the absence of the brown in Blacksburg, Virginia, these data suggest that the gray is the more primitive of the two racial groups and that the brown has been derived from it by mutation. Preliminary evidence (7, 9) indicated that certain gray cultures mutated to the brown but conclusive proof has yet to be obtained.

Endoconidia were formed in varying amounts on all media with or without thiamine. Apparently there is sufficient thiamine in the spores used as inoculum to provide for endoconidial production which occurs within 24 hr. after germination.

When there was a thiamine deficiency on media relatively rich in carbon and nitrogen, chlamydospores did not develop. However, small chlamydospores formed sparsely on water agar which is thiamine-deficient but not necessarily thiamine-free (1). The small amount of thiamine present in agar media relatively high in carbohydrates and nitrogen is probably utilized in carbohydrate metabolism during the four-day lag period preceding chlamydospore formation. Carbohydrate metabolism is greatly reduced in water-agar and may leave traces of thiamine for the slow formation of small chlamydospores.

A deficiency of thiamine in high carbohydrate–nitrogen media retarded radial growth of hyphae. However, radial growth of hyphae was extensive on water-agar. Apparently with high carbohydrate content the available thiamine was blocked or there was a nutritional unbalance. This blockage was often overcome either through adaptation or mutation, after a period of one to two weeks as indicated by the appearance of one-sided radial growth on high carbohydrate–nitrogen thiamine-deficient media although radial growth was not as rapid or extensive as on water-agar.

Chlamydospores in soil extract agar formed more slowly, in fewer numbers, and were smaller than those found on media rich in carbohydrate, nitrogen, and thiamine, or on host tissue *in vivo* or *in vitro*. Requirement for chlamydospore formation of an excess of thiamine, sensitivity to nutrition, the lag period before formation, and the paucity and small size on soil or soil extracts indicate that these spores are more closely associated with and dependent on the parasitic phase than endoconidia. In nature they are likely to be formed mainly during pathogenesis and not during saprogenesis unaccompanied by a previous parasitic phase. Thus chlamydospore growth and behavior are likely to be more sensitive indicators of physiological processes related to parasitism than endoconidial and mycelial growth and behavior.

References

1. Day, D. Thiamine content of agar. Bull. Torrey Botan. Club, 69 : 11-20. 1942.
2. Lilly, V. G. Private communication.
3. Lilly, V. G. and Barnett, H. L. Physiology of the fungi. McGraw-Hill Book Company, Inc., New York. 1951.

4. LILLY, V. G. and BARNETT, H. L. Utilization of sugars by fungi. West Va. Univ. Agr. Expt. Sta. Bull. 362*T*. 1953.

5. LILLY, V. G., BARNETT, H. L., and ANDERSON, B. G. The effects of allithiamine on some thiamine-requiring organisms. Science, 118 : 548-549. 1953.

6. STEINBERG, R. A. Growth on synthetic nutrient solutions of some fungi pathogenic to tobacco. Am. J. Botany, 37 : 711-714. 1950.

7. STOVER, R. H. The black rootrot disease of tobacco. I. Studies on the causal organism *Thielaviopsis basicola*. Can. J. Research, C, 28 : 445-470. 1950.

8. STOVER, R. H. The black rootrot disease of tobacco. II. Physiological specialization of *Thielaviopsis basicola* on *Nicotiana tabacum*. Can. J. Research, C, 28 : 726-738. 1950.

9. STOVER, R. H. Physiological specialization and mutations in *Thielaviopsis basicola*. Abstr. Phytopathology, 41 : 34. 1951.

10. TREZZI, G., BERTINI, S., and LAPRIENO, N. Utilization of some carbonium and nitrogen compounds by *Thielaviopsis basicola*. Agr. ital. (Pisa), 9 : 36-38. 1954.

A SERIES OF BOGS ACROSS QUEBEC FROM THE ST. LAWRENCE VALLEY TO JAMES BAY[1]

By J. E. Potzger[2] and Albert Courtemanche[3]

Abstract

The study includes 19 bogs between 45° 07′ N. and 51° 59′ N. (Fig. 1), spaced at about 50 mile intervals from the St. Lawrence valley across the Laurentian Shield to James Bay (Jack River). Plane service made possible selection of excellent bogs for boring in wilderness regions. The plane landed on lakes near the bogs selected. Up to lat. 47° N. five major climatic changes are recognized for Quebec and are referred to as Q-1 to Q-5. The pollen profiles suggest that an initial, pronounced warm period (Q-1) (correlative with the Lake Timiskaming retreat) followed by cooling (Q-2), very likely also accompanied by local glaciation (correlative with the Cochrane halt), prevailed from the St. Lawrence valley to Lac Soscumica bog (50° 39′ N.). The initial warm period is marked by prominent pine peaks accompanied by an impressive minor oak peak. During the major xerothermic period (Q-3), all bogs record a very long and prominent pine climax, with replacement of *Pinus banksiana* by the white–red pine group up to the Lacroix River bog (49° 02′ N.). From Clova (48° 07′ N.) to Jack River bog (51° 59′ N.) jack pine replaced red–white pine, while the upper half of the profile accumulated. This shows jack pine with a striking bimodal pattern of representation. Also, north of Clova, jack pine formed an important association with *Picea mariana* during the more recent past, introducing the forest type which prevails up there today. From the St. Lawrence valley (45° 07′ N.) to the lower edge of the Shield at Saint-Lin (45° 55′ N.) white–red pine held an important place in the forest cover up to the present (represented by the close of the bog mat). The single most striking feature of the study is that red–white pine penetrated as important forest associates to the Rupert River (51° 28′ N.). No doubt white–red pine extended their range northward during the prominent warm–dry period (Q-3) because their highest representation appears in the lower levels of bogs. The great change in vegetation type, with more emphasis on boreal species (*Picea mariana* and *Pinus banksiana*), from Clova (48° 07′ N.) northward to James Bay suggests the Cochrane oscillation influence and subsequent retreat during the ensuing major xerothermic period (Q-3). This period had probably waned by the time the forests were able to invade the James Bay region, thus giving rise to a rather monotonously changeless forest history from lat. 50° 54′ northward (Q-5). Local glaciation is indicated in the bog from Mont Tremblant (Bog 14), where forest history began during the xerothermic period. Fig. 2 presents the highest percentage attained by species at a given latitude, which at a glance divides the species according to latitudinal preference, suggesting temperature control.

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Since the region about James Bay was covered by the sea following northward wasting of the ice, forest history here begins later than in the areas located above elevation of submergence. Radiocarbon dating of the peat from the bottom level of a bog near Rupert River (51° 27′ N., 78° 32′ W.) places the beginning of organic sediment accumulation at 2350 ± 200 years. This marks the time when marine waters withdrew from the region. Hemlock must have been quite abundant northward to Lac Shaw bog (46° 19′ N.), but reached a pollen representation of 7% even at Lac Mazanaskwa bog (47° 07′ N.). The decline of the white–red pine group on the northern half of the Shield suggests that the muskeg condition north of Clova is due to more recent paludification and more moist climate following the warm–dry period (Q-3). This conclusion is also supported by the prominent rise of *Picea mariana*. Evidence of tundra conditions was found only in bogs 1, 3, 6, 9, 10, and 12.

Introduction

The organic sediments of bogs and lakes in Quebec are continuing to contribute valuable records on forest history and climatic changes during late-glacial and postglacial times. Into the picture enter also various local modifications of climate with variations in successional trends, marking pollen profiles as diagnostic as actual forest study.

Quebec with its great expanse of land surface is in contact with continental and oceanic climatic influences, differing in this respect from other localities where pollen studies were made. Into its physiographic history enter ancient sea bottoms, great plains of former glacial border lakes, and important moraines—rugged, steep slopes cut by moving ice or flowing water into pre-Cambrian rocks, sandy plains, and fertile valleys.

Its vast expanse of land extends from southern broadleaved forest regions in the St. Lawrence valley to the bleak tundra along the borders of Hudson Bay. A great center of continental ice mass left its imprints on the topography and local glaciations add problems to a correct interpretation of the past. Dresser and Denis (4) say: "Of great importance in fashioning the present topographic surface was Pleistocene glaciation."

Here isolated pollen studies are hopelessly submerged by the great expanse and by ramifications of controlling influences on forest history. Thus Quebec demands magnitude in pollen investigations. The seemingly extensive studies by Potzger (12) of 19 bogs ranging from the Gaspé peninsula and Saguenay valley to the lower slopes of the Laurentian Shield, and the more localized investigations in the Mont Tremblant Park area by Potzger and Courtemanche (13), almost immediately demanded systematic line transect studies northward to determine "tendencies" in forest behavior and changes.

The present study may therefore be termed an orderly expansion of the plan to increase magnitude of coverage in conformity with magnitude of land area, a land area with complex physiographic history whose centers of continental ice were the chief control of forest migration southward to the Ohio River and no doubt beyond. Thus the Province of Quebec helped to make history in pollen analysis when in July 1953 it sent a team of scientists northward by plane to James Bay to sample a long series of bogs extending a distance of 500 miles from the St. Lawrence valley to James Bay.

Geology and Physiography

The St. Lawrence lowland has no doubt experienced its greatest modification during the invasion by the Champlain Sea, when land up to 700-ft. elevation was submerged. It also became a barrier to forest invasion of the valley itself and of the Shield to the north. To-day it is a vast level lowland bordered on the south by the Appalachian outliers or highlands and on the north by the southern border or escarpment of the Laurentian Shield. To quote Dresser and Denis (4) "The Laurentian plateau comprises more than 9/10 of the entire area of the Province. It is an immense plateau with an uneven rolling surface. In Quebec, the elevation over most of the plateau varies between 800 and 2,000 feet. The Southern edge of the plateau is well marked by an escarpment."

To the north a glacial lake, Barlow-Ojibway, fringed the southern end of James Bay and extended southward to Senneterre and Lac Témiscamingue, giving rise to silty plains which maintain these characteristics to the present. The fringing lowlands bordering James Bay and Hudson Bay were later submerged by the sea. All of these geologic happenings influenced forest migrations independently of climatic changes.

Methods

Field Work

The specific aim was to run a transect line of bogs which would include at least three of the most conspicuous physiographic features of western Quebec, i.e. the St. Lawrence lowland (Champlain Sea) with the northern border of this sea (Saint-Lin bog), the Laurentian Shield, and the plain of former lake Barlow-Ojibway. The completed plan of study includes 19 bogs, ranging from 45° 07′ N. to 51° 59′ N. They were spaced at about 50 mile intervals from the St. Lawrence valley to James Bay (Fig. 1). It is impossible to cover such an extensive area adequately by land or by river because of the rugged terrain of the Shield and roadless areas of the northern wilderness. Topographic maps, too, are inadequate to indicate the most desirable kind of bog, i.e. the kettle type of not too extensive area.

The authors are therefore very grateful to the Service de Biogéographie of the Province of Quebec for having made plane service available both for selection of bogs and also for boring. This aid and arrangement is indeed ideal. Once a bog had been selected and directional notes had been made, the plane landed on a lake nearby and a team of four workers made soundings to locate the deepest part of the basin. In no case were borings made near a central open pool. Potzger and Wilson (17) and Potzger and Courtemanche (13) have found that such locations always yield a truncated lower part of the profile. When all preliminary precautions had been satisfied, borings were made with a Hiller-type borer 17 in. in cylinder length and $1\frac{3}{4}$ in. in width. Samples were taken at 6-in. intervals except at critical bottom or top layers where samples were at times taken at 1-in. intervals.

The outer layer of the cores was cut away with a pocket knife and a sample not over one half inch in depth was removed from the inner part of the core.

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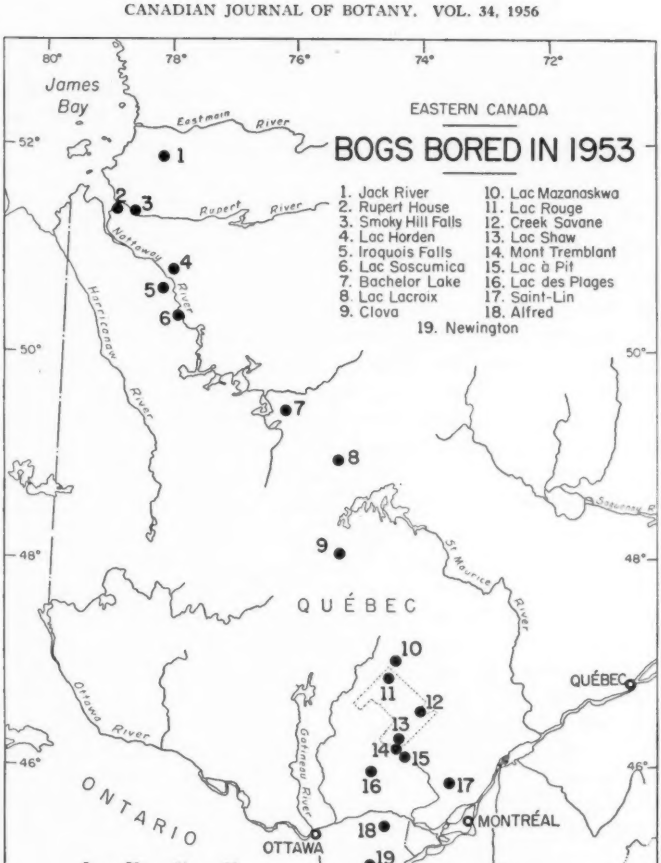

FIG. 1. Map showing area sampled.

The samples were collected in waxed paper, accurately marked as to level, and the peat was later transferred to labeled vials, which were then stoppered securely.

In the Laboratory

Since most of the peat separated readily, the simple Geisler (8) alcohol method was used in preparation of slides. Erdtman (5) KOH procedure was used with samples not readily deflocculated. As a whole, 150 pollens of trees were counted at each foot-level, but all pollens of shrubs and herbaceous plants and spores were tabulated (Table I). Staining was, as usual, with gentian violet, and glycerin jelly served as mounting medium.

TABLE I

NUMBERS OF POLLEN GRAINS OF SHRUBS AND HERBS TABULATED DURING COUNTING OF THE STATED NUMBER OF TREE POLLENS

Bogs	Foot-levels	No. tree pollens	Shrubs				Herbaceous plants							Pteridophytes				
			Alnus	*Betula glandulosa*	*Corylus*	Ericaceae	*Artemisia*	Chenopodiaceae	Compositae	Cyperaceae	Gramineae	*Nymphaea*	*Typha*	*Dryopteris*	*Isoëtes*	Lycopodiaceae	*Osmunda*	Unknown spores
1. Jack River	Surf.	150	16	2	.	1	.	.	2	3	9	.	.	.	.	1	.	.
	½′	"	3	.	1	1	.	1	.	6	4	.	.	.	.	4	.	.
	1′	"	5	.	.	6	.	.	.	2	4	.	.	.	.	.	.	.
	2′	"	4	1	1	.	.	.	.	3	.	.	.	.	.	2	.	3
	3′	"	6	1	.	.	.	.	.	1	.	.	.	.	.	6	.	1
	4′	"	3	1	.	.	.	.	.	4	8	.	.	.	.	13	.	.
	5′	"	8	1	.	2	1	.	.	3	4	2	.	.	.	1	.	.
	6′	"	5	.	.	.	.	.	.	27	8	1	.	.	.	3	.	.
	7′	"	3	.	1	.	1	.	.	6	22	3	.	.	.	2	.	.
	8′	"	4	3	.	.	.	.	.	21	15	4	.	.	.	.	.	.
	9′	"	3	1	5	.	.	1	.	27	34	.	.	.	.	.	.	.
	9′ 1″	"	21	11	.	.	1	.	2	6	50	1	.	.	.	1	5	1
	9½′	"	3	6	.	.	.	1	.	.	2	.	.	.	.	.	.	.
2. Rupert House	Surf.	150	.	.	.	7	.	.	.	4	.	.	1	.	.	.	.	.
	4″	"	2	.	.	3	.	1	.	1	.	.	.	.	.	.	.	.
	1′	"	5	4	1	15	.	1	.	9	2	.	.	.	.	.	.	.
	2′	"	3	2	.	3	.	.	.	3	3	.	.	.	.	5	.	1
	3′	"	1	.	1	.	.	1	3	18	5	.	1	.	.	4	.	2
	4′	"	1	.	.	1	.	.	.	6	3	.	.	.	.	.	.	.
	5′	"	1	2	.	.	.	.	.	4	1	.	.	.	.	.	.	.
	5½′	"	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	6′	100	1	1	1	.	.	.	1	1	2	.	.	.	.	.	.	.
	6½′	150	2	.	1	.	.	1	.	2	1	.	.	.	.	.	.	.
3. Smoky Hills Falls	Surf.	150	2	2	1	8	.	.	2	8	9	.	.	.	.	13	.	2
	½′	"	3	1	.	3	.	.	.	.	2	.	.	.	.	.	.	.
	1′	"	1	1	1	2	.	.	1	.	.	.	.	.	.	1	.	.
	2′	"	2	1	1	5	.	.	.	.	.	.	.	.	.	1	.	1
	3′	"	.	1	1	6	.	.	.	.	3	.	.	.	.	12	.	1
	4′	"	3	2	.	4	.	.	.	1	1	.	.	.	.	3	.	.
	5′	"	1	1	.	5	.	.	.	.	1	.	.	.	.	17	.	.
	6′	"	.	1	.	3	.	.	.	3	1	.	.	.	.	.	.	.
	7′	"	2	2	.	3	.	.	.	.	1	.	.	.	.	2	.	1
	8′	"	1	.	.	2	.	.	.	1	.	.	.	.	.	.	.	.
	8′ 9″	100	4	1	.	.	.	.	.	10	14	.	.	.	.	.	.	.
	9′ 1″	"	2	4	.	.	.	.	2	308	69	.	.	.	.	.	.	.
	9½′	"	3	4	.	.	.	1	.	17	31	.	.	.	.	.	.	.
	10′	"	1	3	.	.	.	1	1	3	6	.	.	.	.	2	.	.
4. Lac Horden	Surf.	150	6	1	1	1	.	2	5	3	6	.	.	.	.	3	.	.
	½′	"	4	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.
	1′	"	2	1	.	1	.	.	.	4	1	.	.	.	.	4	.	.
	2′	"	.	5	.	.	.	.	.	1	.	.	.	.	.	.	.	.
	3′	"	3	.	.	2	.	.	.	2	.	.	.	.	.	.	.	.
	4′	"	.	.	1	2	.	.	.	4	3	.	.	.	.	3	.	.
	5′	"	1	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	6′	"	.	.	.	.	.	.	.	1	.	.	.	.	.	2	.	.
	7′	"	.	1	.	.	.	1	.	3	1	.	.	.	.	.	.	1
	8′	"	1	.	.	.	.	.	.	1	1	.	.	.	.	3	.	.
	9′	"	.	1	.	1	.	.	.	2	.	.	.	.	.	4	.	.
	10′	"	1	2	.	2	.	.	.	1	2	1	.	.	.	7	.	.
	11′	160	6	5	.	.	.	2	.	.	3	.	.	.	.	.	.	.
	12′	150	3	2	.	1	.	.	.	19	1	.	.	.	.	3	.	1
	13′	"	1	3	.	.	.	.	2	31	4	.	.	.	.	.	.	.
	14′	"	.	6	.	.	.	.	.	3	2	.	.	.	.	.	.	.
5. Iroquois Falls	Surf.	150	3	1	.	.	.	1	3	4	4	.	1	.	.	.	.	.
	½′	"	1	.	.	3	.	.	.	.	3	.	.	.	.	.	.	.
	1′	"	1	.	.	2	1	.	.	2	5	.	.	.	.	3	.	.
	2′	"	3	.	1	1	.	.	.	3	2	.	.	.	.	4	.	.
	3′	"	6	2	1	4	1	.	.	6	5	.	.	.	.	1	.	.
	4′	"	4	.	.	4	.	.	.	2	1	.	.	.	.	8	.	.
	5′	"	7	2	1	2	.	.	1	1	4	.	.	.	.	3	.	.
	6′	"	6	4	.	.	.	.	.	16	11	.	.	.	.	.	.	.
	7′	"	2	1	1	.	.	.	1	14	15	.	.	.	.	1	.	.
	8′	"	1	.	1	.	.	.	.	11	6	.	.	.	.	1	.	.
	9′	"	.	2	1	.	.	.	1	22	7	.	.	.	.	1	.	1
	9′ 9″	50	.	1	.	.	.	.	.	6	13	.	.	.	.	.	.	.

TABLE I (*Continued*)

NUMBERS OF POLLEN GRAINS OF SHRUBS AND HERBS TABULATED DURING COUNTING OF THE STATED NUMBER OF TREE POLLENS

Bogs	Foot-levels	No. tree pollens	Shrubs				Herbaceous plants							Pteridophytes				
			Alnus	*Betula glandulosa*	*Corylus*	Ericaceae	*Artemisia*	Chenopodiaceae	Compositae	Cyperaceae	Gramineae	*Nymphaea*	*Typha*	*Dryopteris*	*Isoetes*	Lycopodiaceae	*Osmunda*	Unknown spores
6. Lac Soscumica	Surf.	150	2	3	1	.	.	.	1	1	3	.	.	.	.	.	.	.
	3″	"	1	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
	½′	"	2	.	.	.	.	.	.	3	6	.	.	.	.	.	.	.
	1′	"	1	.	.	.	.	.	.	.	7	.	.	.	.	.	.	.
	2′	"	.	.	2	.	.	1	.	.	5	.	.	.	.	.	.	.
	3′	"	.	.	.	.	.	.	.	2	3	.	.	.	.	.	.	.
	4′	"	.	.	.	.	.	.	.	3	3	.	.	.	.	.	.	1
	5′	"	.	.	.	.	.	.	1	2	4	.	.	.	.	.	.	.
	6′	"	.	1	1	.	.	.	.	1	1	.	.	.	.	.	.	1
	7′	"	.	1	1	1	.	.	.	2	4	.	.	.	.	.	.	.
	8′	"	1	.	.	.	.	.	1	3	6	.	.	.	.	.	.	1
	9′	"	1	.	.	.	.	.	.	.	5	1	.	.	.	.	.	1
	10′	"	2	3	.	2	.	.	.	3	8	.	.	.	.	79	.	.
	11′	"	.	.	.	.	.	.	1	.	5	.	.	.	.	15	.	2
	12′	"	1	3	.	.	.	.	.	3	1	.	.	1	.	.	.	1
	12′ 3″	"	1	2	.	.	.	1	.	1	8	.	.	.	.	.	.	1
	12½′	"	.	7	.	.	.	.	.	2	2	.	.	.	.	.	.	.
	12′ 9″	25	1	375	.	.	.	.	.	1	4	.	.	.	.	2	.	.
	13′	15	2	248	1	.	.	.	.	1	6	.	.	.	2	.	.	3
	13½′	100	1	.	.	.	.	1	2	1	7	.	.	.	.	.	.	.
7. Bachelor Lake	Surf.	150	1	1	.	11	.	.	1	2	1	.	.	.	.	.	.	.
	½′	"	2	4	.	4	.	.	1	3	1	.	.	.	.	.	.	.
	1′	"	2	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.
	2′	"	6	6	.	2	.	.	3	10	5	.	.	.	.	.	.	.
	3′	"	5	2	1	1	.	.	.	.	3	.	.	.	.	.	.	.
	4′	"	7	3	.	7	.	.	.	5	.	.	.	.	.	6	.	1
	5′	"	1	1	4	.	.	.	.	.	3	.	.	1	.	2	.	.
	6′	"	1	.	.	.	.	.	.	.	3	.	.	.	.	2	1	2
	7′	"	.	.	1	.	.	.	.	.	1	.	.	.	.	.	.	.
	8′	125	2	.	.	.	.	.	.	12	7	.	.	.	.	.	.	.
	8′ 1″	97	.	.	.	.	.	.	.	61	19	.	.	.	.	2	.	.
	8½′	75	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	9′	150	3	3	.	.	.	.	.	35	13	.	3	1	.	.	.	.
	9′ 2″	50	.	1	.	.	.	.	.	4	5	.	.	.	.	1	.	.
8. Lac Lacroix	Surf.	150	3	1	.	3	.	.	3	2	2	.	.	.	.	3	.	.
	½′	"	4	1	.	.	.	.	.	3	1	.	.	.	.	.	.	.
	1′	"	.	.	.	2	.	.	.	1	1	.	.	.	.	1	.	.
	2′	"	2	3	.	.	.	1	2	.	2	.	.	.	.	.	.	.
	3′	"	2	1	1	.	.	.	.	.	3	.	.	.	.	.	.	.
	4′	"	3	4	1	.	.	.	.	2	1	.	.	.	.	1	.	.
	5′	"	.	3	.	1	.	.	.	.	.	.	.	.	.	.	.	.
	6′	"	1	.	.	2	.	.	1	1	.	.	.	.	.	5	.	.
	7′	"	1	3	1	3	.	.	.	.	.	.	.	.	.	4	.	.
	8′	"	1	1	.	1	.	.	2	.	1	.	.	.	.	.	.	.
	9′	"	2	.	.	2	.	.	.	4	.	.	.	.	.	2	.	.
	10′	"	.	1	.	.	.	.	.	3	2	.	.	.	.	.	.	2
	11′	200	1	.	.	.	1	.	.	.	2	.	.	.	.	.	.	.
	12′	"	4	5	.	.	.	.	1	2	4	.	.	.	.	2	.	.
	13′	150	9	9	.	.	.	.	.	.	.	1	.	.	.	.	.	.
	14′	"	5	18	.	.	.	.	.	.	2	.	.	.	.	.	.	.
	15½′	"	4	3	1	1	.	.	.	1	3	.	.	.	.	.	.	.
	16′	"	7	5	.	1	1	1	1	3	4	.	.	.	.	1	.	.
	17′	200	5	5	.	1	.	1	.	6	3	1	.	.	.	1	.	1
	18′	150	5	.	.	1	2	1	.	1	1	.	.	.	.	.	.	.
	18½′	"	24	17	.	.	.	.	1	6	1	.	.	.	.	.	.	.
	19′	"	20	11	1	.	.	.	5	3	5	.	.	.	.	.	.	.
	19′ 4″	"	16	14	.	.	.	.	3	1	11	.	.	.	.	.	.	1
	19½′	100	14	4	.	1	.	.	.	.	3	.	.	.	.	.	.	.
9. Clova	Surf.	150	.	7	.	1	.	.	.	11	4	.	.	.	.	1	.	.
	½′	"	1	5	1	29	.	.	2	1	4	.	.	.	.	.	.	.
	1′	"	3	2	.	.	.	.	1	1	1	.	.	.	.	.	.	.
	2′	"	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	3′	"	.	4	.	1	.	.	.	3	1	.	.	.	.	1	.	.
	4′	"	2	2	.	1	.	.	.	1	1	.	.	.	.	.	1	.
	5′	"	1	6	.	.	.	.	.	.	.	.	.	1	.	.	.	.
	6′	"	5	3	.	.	.	.	.	.	2	.	.	.	.	.	.	.
	6½′	"	3	2	.	.	.	.	.	6	9	.	.	.	.	1	.	.
	7′	"	2	4	.	.	.	.	.	41	2	.	.	.	.	.	.	.
	8′	"	11	7	.	2	.	.	3	3	1	.	.	.	.	1	.	.

TABLE I (*Continued*)

NUMBERS OF POLLEN GRAINS OF SHRUBS AND HERBS TABULATED DURING COUNTING OF THE STATED NUMBER OF TREE POLLENS

Bogs	Foot-levels	No. tree pollens	Shurbs				Herbaceous plants							Pteridophytes				
			Alnus	*Betula glandulosa*	*Corylus*	Ericaceae	*Artemisia*	Chenopodiaceae	Compositae	Cyperaceae	Gramineae	*Nymphaea*	*Typha*	*Dryopteris*	*Isoetes*	Lycopodiaceae	*Osmunda*	Unknown spores
9. Clova (*Continued*)	9′	"	8	8	.	2	.	.	.	3	.	1	.	.	.	.	.	.
	10′	"	4	13	.	.	.	.	.	1	1	.	.	.	.	.	.	.
	11′	"	8	16	.	.	.	.	.	4	3	2	.	.	.	.	.	.
	12′	"	13	8	.	.	.	1	.	3	1	.	.	.	.	.	.	.
	13′	"	8	4	.	2	.	.	.	1	1	.	.	1	.	.	.	.
	14′	"	14	16	.	1	.	.	1	3	5	.	.	.	.	.	.	.
	15′	"	7	2	1	.	.	.	.	.	1	1	.	.	.	.	.	.
	16′	"	6	2	.	.	.	.	.	.	.	.	1	.	.	.	.	.
	17′	"	1	8	1	1	.	1	.	1	2	.	.	.	.	.	.	.
	18′	"	5	5	1	.	.	1	2	.	4	4	.	.	1	2	.	.
	19′	"	11	3	.	.	.	.	.	3	3	4	.	.	.	1	.	.
	20′	"	30	30	1	.	.	.	2	3	26	.	1	.	5	3	.	4
	20′ 2″	100	8	6	.	.	.	.	1	5	6	.	.	.	.	.	.	.
	20½′	"	15	10	3	.	.	1	2	3	9	.	.	.	.	.	.	.
	21′	"	7	4	.	.	.	1	2	.	11	.	.	.	.	.	.	.
	21½′	150	7	11	1	.	.	.	.	.	9	.	.	.	.	.	.	.
10*a*. Mazanaskwa (A)	Surf.	200	3	6	.	3	.	1	1	3	.	.	.	.	.	.	.	.
	½′	"	3	5	.	.	.	.	2	1	3	.	.	.	.	31	.	.
	1′	150	3	6	.	.	.	1	4	.	1	.	.	.	.	2	.	.
	2′	"	3	15	2	4	.	.	1	.	7	.	1	.	.	.	.	.
	3′	200	4	28	1	.	.	.	.	.	.	.	.	.	.	1	.	.
	4′	150	.	2	2	.	.	.	.	.	3	.	.	.	.	8	.	.
	5′	200	.	6	.	.	.	.	.	.	.	.	.	.	.	1	.	.
	6′	175	.	4	1	.	.	.	.	2	1	.	.	.	.	.	.	.
	7′	150	1	5	.	.	.	.	.	1	1	.	.	.	.	1	.	.
	8′	200	2	6	.	.	.	.	.	1	.	.	1	.	.	2	.	.
	9′	"	2	19	.	.	.	.	2	.	.	.	.	.	.	.	.	1
10*b*. Mazanaskwa (B)	10′	150	3	5	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	11′	"	3	17	.	.	.	.	.	.	.	.	.	.	.	1	.	1
	12′	200	2	25	.	.	.	.	1	1	1	.	.	.	.	.	.	.
	13′	150	.	6	.	.	.	.	.	1	3	.	.	.	.	.	.	.
	14′	200	.	5	.	.	.	.	.	.	1	.	.	.	.	1	.	.
	15′	"	1	13	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	16′	225	1	2	2	.	.	.	.	3	1	3	.	.	.	.	.	.
	17′	150	1	9	.	1	.	.	.	.	.	5	.	.	.	.	.	.
	18′	"	.	7	.	.	.	.	.	.	1	1	.	.	.	.	.	.
	19′	200	2	8	.	.	.	.	.	1	.	.	.	.	.	.	.	.
	20′	"	1	5	.	.	.	.	.	1	.	.	.	.	.	1	.	.
	21′	"	2	5	.	1	.	.	1	1	.	.	.	.	.	.	.	.
	22′	150	.	3	.	1	.	.	.	2	2	2	.	.	.	2	.	.
	23′	"	13	2	.	.	.	1	.	2	4	.	.	.	1	.	.	.
	24′	200	6	3	.	1	.	.	.	.	6	6	.	.	.	2	.	1
	25′	100	15	2	.	1	.	.	3	1	143	.	.	.	40	1	.	.
	25½′	150	10	7	.	1	.	.	2	2	48	.	.	.	312	3	.	.
	26′	100	18	3	.	.	.	.	2	.	152	.	.	.	308	4	.	.
11. Lac Rouge	Surf.	150	12	8	.	.	.	.	3	.	.	.	.	.	.	.	.	.
	½′	"	4	1	.	.	.	.	.	.	.	.	.	.	.	.	.	1
	1′	"	26	1	5	3	.	.	1	.	.	.	.	.	.	.	.	3
	2′	"	11	1	.	1	.	.	.	2	.	.	.	.	.	.	.	.
	3′	"	7	.	.	3	.	.	.	1	.	.	.	.	.	1	.	1
	4′	"	32	2	7	.	.	.	1	3	.	.	1	9	.	.	.	3
	5′	"	14	2	3	.	.	.	.	.	.	.	.	2	.	.	.	3
	6′	"	25	5	2	.	.	.	.	.	.	.	.	2	.	.	.	.
	7′	"	22	1	5	.	.	.	.	2	.	.	1	4	.	.	.	.
	8′	"	17	3	1	.	.	.	.	.	.	.	.	.	.	.	.	.
	9′	"	23	.	4	.	.	.	.	.	.	.	1	4	.	.	.	.
	10′	"	12	.	1	.	.	.	.	.	.	.	.	1	.	.	.	.
	11′	"	16	1	4	.	.	.	.	.	.	.	.	1	.	.	.	.
	12′	"	11	3	6	.	.	.	.	1	.	.	.	2	.	4	.	1
	13′	"	14	1	3	.	.	.	.	2	.	.	2	3	.	.	.	.
	14′	"	6	.	1	.	.	.	.	.	.	.	.	.	.	.	.	2
	15′	"	2	1	1	.	.	.	.	.	.	.	.	2	.	.	.	.
	16′	"	7	.	.	.	.	.	.	.	.	.	.	1	.	.	.	2
	16½′	"	6	.	2	.	.	.	2	.	.	.	.	.	.	.	.	2
	17′	"	7	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.
	18′	"	8	1	2	.	.	.	.	.	.	.	.	.	.	.	.	.
	19′	"	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	20½′	"	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
	21′	"	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.

TABLE I (*Continued*)

NUMBERS OF POLLEN GRAINS OF SHRUBS AND HERBS TABULATED DURING COUNTING OF THE STATED NUMBER OF TREE POLLENS

Bogs	Foot-levels	No. tree pollens	Shrubs				Herbaceous plants							Pteridophytes				
			Alnus	*Betula glandulosa*	*Corylus*	Ericaceae	*Artemisia*	Chenopodiaceae	Compositae	Cyperaceae	Gramineae	*Nymphaea*	*Typha*	*Dryopteris*	*Isoetes*	Lycopodiaceae	*Osmunda*	Unknown spores
12. Creek Savanne	Surf.	150	4	1	.	.	.	.	6	.	3	.	.	.	.	.	.	.
	1′	"	2	1	.	.	.	.	1	.	1	.	.	.	.	.	.	.
	2′	"	3	1	.	.	.	.	.	.	4	.	.	.	.	.	.	.
	3′	"	.	1	1	.	.	.	.	.	.	.	.	.	.	1	.	1
	4′	"	1	.	1	1	.	.	.	.	.	1	.	.	.	.	.	.
	5′	"	.	.	.	.	.	.	1	.	3	.	.	.	.	.	.	.
	6′	"	15	6	.	.	.	.	1	.	.	.	.	.	.	.	.	.
	7′	"	8	13	2	.	.	.	.	.	3	1	.	.	.	.	.	.
	8′	"	7	16	.	1	.	.	2	.	3	1	.	.	.	.	.	.
	9′	"	5	5	.	.	.	.	.	.	4	.	.	.	.	1	.	.
	10′	"	.	5	.	.	.	.	.	1	.	1	.	.	.	.	.	.
	11′	"	.	5	.	.	.	.	.	2	2	.	.	.	.	.	.	1
	12′	"	1	3	.	.	.	.	.	.	.	1	.	.	.	.	.	1
	13′	"	.	.	.	.	.	.	2	.	3	1	.	.	.	.	.	1
	14′	"	.	1	.	2	.	.	1	6	7	.	.	.	.	.	.	2
	15′	"	6	.	1	.	.	.	1	.	4	2	.	1	.	.	.	.
	16′	"	3	.	1	.	.	.	.	.	4	.	.	.	.	.	.	.
	17′	"	2	2	.	.	.	.	1	.	3	8	.	.	.	.	.	.
	18′	"	3	4	2	.	.	.	.	.	10	1	.	.	.	.	.	1
	19′	"	4	4	3	.	.	.	.	.	4	.	1	.	.	.	.	2
	19½′	"	2	19	5	.	.	.	1	3	11	1	.	1	89	.	.	.
	20′	"	.	5	5	.	.	.	.	.	6	.	1	.	.	.	.	2
	20½′	"	4	52	3	2	.	.	.	.	.	.	.	.	.	.	.	.
	20′ 8″	87	4	4	8	.	.	.	3	.	18	.	.	.	.	.	.	.
13. Lac Shaw	Surf.	150	2	.	.	.	.	.	7	.	7	.	.	.	.	.	.	.
	½′	"	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	1′	"	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
	2′	"	.	.	.	.	.	.	.	.	3	.	.	.	.	.	1	.
	3′	"	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	4′	"	1	.	.	2	.	.	.	.	1	5	1	.	.	.	.	.
	5′	"	2	1	.	.	.	.	.	1	3	3	1	1	.	.	.	.
	6′	"	1	.	.	1	.	.	.	.	7	2	1	1	.	.	.	.
	7′	"	1	.	.	.	.	.	.	.	1	.	1	.	.	.	.	.
	8′	"	10	.	6	.	.	.	1	1	6	.	.	1	.	.	.	.
	9′	"	1	.	.	.	.	.	.	.	4	1	.	.	.	.	.	.
	10′	"	.	.	.	.	.	.	.	.	3	1	.	.	.	.	.	.
	11′	"	4	2	3	.	.	.	.	.	2	5	.	.	.	.	.	.
	12′	200	11	.	.	.	.	.	1	.	4	2	1	.	.	1	.	.
	13′	150	8	.	.	.	.	.	.	1	.	1	2	1	.	.	.	.
	14′	"	3	.	.	.	.	.	.	.	.	3	.	.	.	1	.	.
	15′	"	.	.	.	.	.	.	.	.	1	3	.	.	.	.	.	.
	16′	"	1	1	.	.	.	.	.	.	1	1	.	2	.	.	.	.
	17′	"	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.
	18′	"	.	.	.	.	.	.	.	.	.	2	1	.	.	.	.	.
	19′	"	.	.	1	.	.	.	1	1	.	1	1	.	.	.	.	.
	20′	"	1	.	.	.	.	.	.	1	.	1	.	.	.	.	.	.
	21′	"	1	.	2	.	.	.	.	.	1	2	.	.	.	.	.	.
	22′	"	1	1	1	.	.	.	1	.	.	2	.	.	.	.	.	.
	23′	"	3	.	2	.	.	.	.	1	7	3	.	.	3	.	.	.
	24′	"	7	1	.	.	.	1	1	2	67	.	1	.	.	.	.	.
	24′ 6″	"	4	1	2	.	.	.	.	.	39	.	.	.	.	.	.	.
14. Mont Tremblant	Surf.	150	.	.	.	.	.	.	5	9	20	.	.	1.	.	11	.	.
	4″	"	.	1	.	.	.	.	9	23	51	.	.	.	.	.	.	.
	1′	"	.	.	.	.	.	.	1	4	23	.	.	.	.	.	.	2
	2′	"	2	.	1	.	.	.	2	.	13	1	2	5	.	.	.	.
	3′	"	7	5	1	.	.	.	2	1	15	1	2	1	.	.	.	1
	4′	"	5	1	6	.	.	.	.	.	16	1	1	2	.	.	.	1
	5′	"	7	8	1	.	.	1	.	.	6	.	1	.	.	.	.	1
	5½′	"	27	1	1	.	.	.	1	1	2	.	.	.	.	2	.	.
	6′	"	24	2	2	.	.	.	2	1	9	.	.	.	.	.	.	2
	6½′	"	13	.	.	.	.	.	.	.	2	.	.	1	.	.	.	.
	6½′	"	5	.	.	.	.	.	.	.	2	.	.	.	.	1	.	.
	7′	103	10	3	1	.	.	.	1	.	10	.	.	.	.	.	.	.
15*a*. Lac à Pit (A)	Surf.	150	3	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.
	2′	"	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
	3′	"	1	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.
	6½′	"	2	1	3	4	.	.	.	.	1	.	3	.	.	.	.	.

TABLE I (*Continued*)

NUMBERS OF POLLEN GRAINS OF SHRUBS AND HERBS TABULATED DURING COUNTING OF THE STATED NUMBER OF TREE POLLENS

Bogs	Foot-levels	No. tree pollens	Shrubs				Herbaceous plants							Pteridophytes				
			Alnus	*Betula glandulosa*	*Corylus*	Ericaceae	*Artemisia*	Chenopodiaceae	Compositae	Cyperaceae	Gramineae	*Nymphaea*	*Typha*	*Dryopteris*	*Isoetes*	Lycopodiaceae	*Osmunda*	Unknown spores
15*a*. Lac à Pit (A) (*Continued*)	7′	"	1	.	1	.	.	.	.	.	.	.	.	.	.	15	.	.
	8′	"	1	.	.	.	.	.	1	1	.	.	.	1	.	.	.	.
	9′	"	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	10½′	"	2	.	1	.	.	.	.	.	.	.	1	.	.	.	.	.
	11′	"	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	1
	12′	"	2	1	.	.	.	.	1	.	1	.	.	.	.	.	.	.
	13′	200	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	14′	150	1	1	1	.	.	.	.	.	1	.	.	.	.	.	.	.
	15′	"	.	2	.	.	.	.	.	.	.	.	.	.	.	1	.	.
	16′	"	1	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
	17′	"	3	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	18′	"	.	.	.	1	.	.	.	.	.	1	1	.	.	.	.	.
	18½′	"	.	.	.	.	.	.	.	.	1	1	.	.	.	.	.	1
	20′	"	.	2	.	.	.	.	.	2	1	.	.	1	.	.	.	.
	21′	"	.	1	.	.	.	.	.	1	1	.	.	.	.	.	.	.
	22′	"	.	1	.	1	.	.	.	.	3	.	.	.	.	.	.	.
15*b*. Lac à Pit (B)	20′ 6″	150	1	7	.	.	.	.	.	.	1	.	.	.	.	.	.	.
	21′	"	1	11	.	.	.	.	2	.	1	.	.	.	.	.	.	.
	23′	"	1	5	1	.	.	.	.	.	2	.	.	.	.	.	.	.
	24′	"	7	3	.	.	.	.	.	.	3	.	.	.	.	.	.	1
	25′	"	1	1	.	.	.	.	1	.	1	1	.	.	.	.	.	.
	25′ 6″	"	14	10	.	.	.	.	1	.	1	1	.	.	.	.	.	.
	26′	"	3	4	.	.	.	.	.	1	.	1	.	.	.	.	.	.
	27′	"	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	1
	27′ 6″	"	1	3	.	.	.	.	.	.	1	.	.	.	.	.	.	1
	28′	"	3	5	.	.	.	.	1	.	1	1	.	.	.	.	.	.
	29′	"	1	2	.	.	.	.	1	.	1	.	.	.	.	.	.	.
	30′	"	.	.	2	.	.	.	.	.	1	.	.	.	.	.	.	.
	31′	"	3	2	.	.	.	.	.	.	6	1	.	.	.	.	.	.
	31′ 6″	140	12	1	.	.	.	.	6	.	1	3	.	.	.	.	.	.
	32′	150	4	2	1	.	.	.	.	.	5	1	.	.	.	.	.	1
	33′	"	1	1	1	.	.	.	1	.	5	1	.	.	.	.	.	1
	34′	"	5	.	.	.	.	.	.	.	1	.	.	.	.	.	.	1
	34′ 6″	"	4	1	2	.	.	1	2	.	3	.	.	1	.	.	.	1
	35′	126	6	11	2	1	.	.	1	.	5	.	.	.	.	.	.	2
	35′ 6″	100	3	5	2	.	.	.	2	.	4	.	2	.	.	.	.	.
	36′	"	4	3	1	.	.	.	1	.	4	.	1	.	.	.	.	.
16*a*. Lac des Plages (A)	1′	150	1	.	.	4	.	.	4	3	6	.	.	.	.	4	.	.
	2′	"	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.
	3′	"	.	.	.	8	.	.	.	1	.	.	.	.	.	.	.	1
	4′	"	.	.	1	.	.	.	.	.	2	.	.	.	.	.	.	.
	4½′	"	.	.	.	1	.	.	.	.	4	.	.	.	.	.	.	.
	5′	"	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	6′	"	.	.	.	3	.	.	.	.	1	.	.	.	.	1	.	.
	7′	"	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.
	8′	200	.	.	1	1	.	.	1	.	6	.	.	.	.	.	.	1
	9′	150	3	.	1	1	.	.	.	.	3	.	.	.	.	.	1	1
	10′	"	3	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.
	11′	"	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	12′	"	.	.	1	4	.	.	.	.	1	.	.	1	.	.	1	.
	13′	"	1	.	1	1	.	.	.	.	3	.	.	.	.	.	.	.
	14′	"	1	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
	15′	"	.	.	.	.	.	.	.	1	1	.	.	.	.	.	.	.
	16′	"	.	.	.	.	.	.	.	1	1	.	.	.	.	.	.	.
	17′	"	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	18′	"	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
	19′	"	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
	19½′	"	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.
16*b*. Lac des Plages (B)	31′	150	2	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
	32′	"	1	.	.	.	.	.	.	.	1	2	.	.	.	.	.	1
	33′	"	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.
	35′	"	1	.	.	.	.	3	.	.	1	.	.	.	.	.	.	.
	36′	"	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	36½′	"	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
	37′	"	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.
	38′	"	2	.	.	.	.	.	1	.	3	.	.	.	.	.	.	.
	38½′	"	3	.	.	.	.	.	1	.	4	.	1	.	.	.	.	.
	38′ 9″	100	1	1	.	.	.	.	1	.	7	.	1	.	.	.	.	1
	39′	150	.	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.

TABLE I (*Concluded*)

NUMBERS OF POLLEN GRAINS OF SHRUBS AND HERBS TABULATED DURING COUNTING OF THE STATED NUMBER OF TREE POLLENS

Bogs	Foot-levels	No. tree pollens	Shrubs				Herbaceous plants						Pteridophytes				
			Alnus	*Betula glandulosa*	*Corylus*	Ericaceae	*Artemisia*	Chenopodiaceae	Compositae	Cyperaceae	Gramineae	*Nymphaea*	*Typha*	*Dryopteris*	*Isoetes*	Lycopodiaceae	*Osmunda*	Unknown spores
17. Saint-Lin	Surf.	150	2	19	.	33	.	3	15	.	7	.	.	.	.	3	.	.
	½′	"	1	.	1	20	.	1	.	.	4	.	.	.	.	.	.	.
	1′	"	1	2	1	1	.	.	.	.	6	.	.	.	.	1	.	.
	2′	"	3	1	.	1	.	.	.	3	2	.	.	.	.	.	.	1
	3′	"	.	3	.	4	.	1	.	3	.	1	.	.	.	.	.	1
	4′	"	.	.	.	.	.	.	.	1	4	.	.	.	.	.	.	.
	5′	"	3	2	3	1	.	.	.	.	19	.	.	.	.	.	.	.
	6′	"	.	3	.	1	.	.	.	.	10	.	.	.	.	.	.	1
	7′	"	6	.	.	1	.	1	.	1	14	.	.	.	.	.	.	2
	8′	"	.	8	3	.	.	1	.	.	4	.	.	.	.	.	.	.
	9′	"	.	2	2	1	.	.	.	.	3	.	.	.	.	.	.	.
	10′	"	.	.	.	2	.	.	.	.	9	.	.	.	.	.	.	.
	11′	"	.	4	.	1	.	.	.	.	1	.	.	.	.	.	.	.
	12′	"	2	7	.	.	.	.	.	1	3	.	.	.	.	.	.	.
	13′	"	1	.	.	.	.	.	.	2	3	.	.	.	.	.	.	.
	14′	"	3	.	.	.	.	.	.	1	5	.	1	.	.	.	.	.
	15′	"	2	4	1	.	1	.	.	1	5	.	.	.	.	.	.	.
	16′	"	13	16	.	.	.	.	.	13	28	.	6	1	.	.	.	.
	17′	20	1	.	.	.	.	1	.	2	8	.	.	.	.	.	.	.
	17½′	75	.	1	1	.	.	.	6	7	7	2	.	.	.	.	.	.
	19′	"	1	7	1	.	.	.	1	10	4	.	.	.	.	.	.	.
	21′	150	1	19	.	.	.	.	.	1	6	.	.	.	.	.	.	.
18. Alfred	Surf.	200	4	.	.	5	.	1	13	1	16	.	2	.	.	.	.	.
	½′	150	2	12	.	2	.	.	18	10	25	.	.	.	2	.	.	.
	1′	200	2	.	1	8	.	.	.	.	1	.	.	.	.	.	.	.
	2′	150	1	.	.	.	.	.	.	2	2	.	.	.	.	1	.	.
	3′	"	2	.	.	1	.	.	.	.	5	.	.	.	.	1	.	1
	4′	"	1	.	.	1	.	.	.	2	2	.	.	.	.	.	.	.
	5′	"	2	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
	6′	"	1	8	.	.	.	.	2	4	5	.	.	.	.	.	.	1
	7′	"	.	.	2	.	.	.	.	3	7	.	.	.	.	.	.	1
	8′	"	1	1	.	2	.	.	.	9	7	.	.	.	.	.	.	.
	9′	"	.	.	1	.	.	.	.	4	4	.	.	.	.	.	.	.
	10′	"	2	.	.	.	.	.	.	3	4	.	.	.	.	.	.	1
	11′	"	1	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.
	12′	200	1	.	.	1	.	.	.	5	9	.	1	.	.	2	1	.
	13′	150	.	.	4	1	.	.	.	5	5	.	1	.	.	1	1	1
	14′	200	1	.	2	1	.	.	.	1	2	.	.	.	.	.	1	.
	15′	150	4	3	3	1	.	.	1	1	14	.	.	.	.	.	.	.
	16′	200	3	.	.	.	.	.	1	.	12	.	3	.	.	.	.	.
	17′	150	.	2	1	1	.	.	1	.	2	.	1	.	.	.	.	.
	18′	"	2	3	3	.	.	.	1	1	3	.	.	.	.	.	.	1
	19′	200	3	3	.	1	.	.	.	4	5	.	.	.	.	.	.	.
	20′	150	.	3	1	.	.	.	1	.	6	.	.	1	.	.	.	.
	20′ 3″	"	.	.	.	.	.	.	1	6	15	.	3	.	.	.	.	.
	20½′	25	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.
19. Newington	Surf.	150	3	10	1	2	.	1	19	.	9	.	1	.	.	7	.	2
	½′	"	4	3	1	6	.	2	9	1	25	.	1	.	.	.	.	.
	1′	"	1	2	.	2	.	.	.	.	5	.	.	.	.	.	.	.
	2′	"	2	1	.	1	.	.	.	.	1	.	.	.	.	.	.	1
	3′	"	.	4	.	3	.	.	.	1	3	.	.	.	.	25	.	.
	4′	"	.	2	1	1	.	.	.	.	1	.	.	.	.	5	.	3
	5′	"	.	2	.	2	.	.	1	.	1	.	1	1	.	9	.	.
	6′	"	4	.	.	4	.	.	.	.	4	.	.	.	.	2	.	.
	7′	"	4	3	1	2	.	.	.	.	5	.	.	.	.	.	1	.
	8′	"	1	.	.	3	.	.	.	1	8	.	.	.	.	.	.	2
	9′	"	2	1	.	.	.	.	.	1	8	.	.	.	.	.	1	.
	10′	"	1	1	2	.	.	.	1	2	12	.	.	.	.	.	.	.
	11′	"	.	3	.	.	.	.	.	3	12	.	1	.	.	.	.	.
	12′	"	.	.	1	3	.	.	.	1	6	.	.	1	.	.	.	1
	13′	"	1	1	.	2	.	.	1	.	6	.	1	2	.	.	.	.
	14½′	"	2	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.
	15′	"	4	5	.	.	.	.	1	1	3	.	1	.	.	.	.	.
	16′	"	1	4	.	3	.	.	.	.	6	.	.	1	.	1	.	4
	17′	"	1	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	18′	"	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.
	19′	"	2	9	.	.	.	.	1	.	.	.	.	.	.	.	.	.
	19½′	"	.	5	2	.	.	.	.	.	3	.	1	.	.	.	.	.
	20′	"	.	1	.	.	.	.	.	1	2	.	5	.	.	.	.	.
	20′ 8″	"	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	21′	"	.	2	.	.	.	.	2	.	.	.	2	.	.	.	.	.
	21¼′	"	1	3	.	.	.	.	.	.	21	.	2	.	.	.	1	.

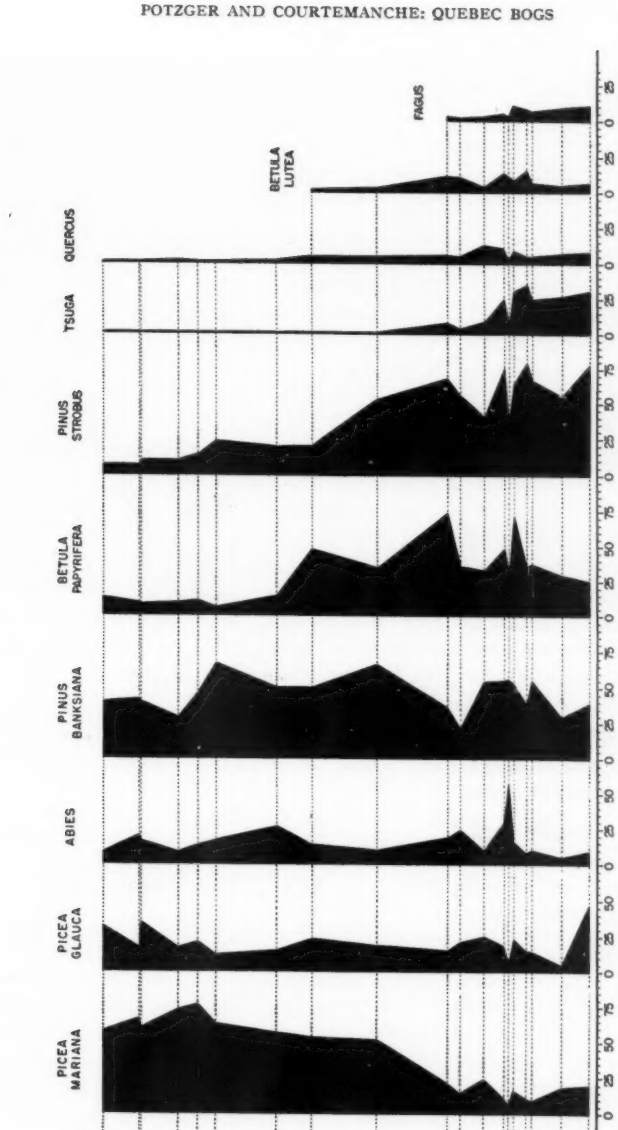

FIG. 2. Highest pollen percentages attained by various genera and species at different latitudes.

Pine pollens are differentiated into three groups as jack pine, 34 μ depth of tube cell and 59 μ total width of expanded grain as maximum size. White–red pine, 38.5 μ for depth of tube cell and 62.5 μ total width of expanded grain, or larger. Pine pollens with 35 to 38 μ depth of tube cell and 58 to 62 μ total width of grain are designated as "pine intermediate".

A previous preliminary study of *Betula* from Quebec, based on measurements of 50 pollen grains for each collection, gave a very small overlap in size between *Betula glandulosa* (21 μ or less), *B. papyrifera* (22 to 26 μ), *B. lutea* (26.5 μ or larger). The material was boiled in alcohol and mounted in glycerin jelly. Our differentiation of *Betula* is based on these measurements. Additional size studies in 1955 make this differentiation less definite. It appears that only collections from many trees in the same region will give satisfactory data. In limited collections any one tree may influence real tendencies too much.

Shrubs and herbs are not included in the pollen profiles, but are listed in Table I as total number of grains counted. This appears to be significant in determining possible tundra conditions during early sedimentation. Besides the species and genera listed in Table I, the following occurred as isolated grains at a few foot-levels of the 19 bogs: *Acer spicatum*, *Cornus*, *Nemopanthus*, *Drosera*, *Epilobium*, *Silene*, and *Selaginella*.

At the Smoky Hills Falls bog (Bog 3), peat was collected in bulk at the sharp contact line between rock flour and organic matter. The multiple-shot samples were carbon dated by Lamont Geological Laboratory. The date given was 2350 $\pm$ 200 years (Lamont No. 219).

Potzger (12) recognized five major climatic changes in Quebec, some of which are especially well shown in the Quebec pollen profiles. In the future they will be referred to as:

Q-1. Initial warm period.
Q-2. Colder, moist.
Q-3. Maximum warmth, dry (xerothemic period).
Q-4. Warm, moist.
Q-5. Colder, moist.

Forests of the Present

There is no better description of forest types in Canada than the one by Halliday (9). His descriptions will again be accepted as authentic for the region where the bogs are located.

On a collecting trip, where time and cost of service are factors of major significance, detailed study of forest composition is impossible, but limited notes were taken on the forests surrounding the bogs. In the muskeg terrain north of Clova, forest associations are simple, and as a whole jack pine is limited to rocky outcrops, there constituting almost pure stands (Fig. 3). Birch and quaking aspen have a very sparse representation northward of Clova. Muskeg terrain had a preponderance of black spruce with scattered tamarack.

FIG. 3. Jack pine stand at lat. 51° 59′ N.

Description of Bogs

Newington Bog (45° 07′ N., 74° 58′ W.; alt. *ca.* 325 ft.). This is an oval-shaped bog in the Champlain Sea bottoms. It is located near the town of Newington on the farm of Mr. W. N. Eamer. The completely closed central area has been invaded by numerous young black spruce. Eight sample borings were taken. The final boring was made about a quarter mile from the border near the central region.

Alfred Bog (45° 30′ N., 74° 48′ W.; alt. *ca.* 160 ft.) is a very extensive peat deposit near the town of Alfred. The boring was made from the south side along a winter road about one mile north and a half mile east from the entrance. The surface of the bog is covered quite extensively by black spruce forest with tamarack and some fir except for occasional open areas where *Ledum*, *Chamaedaphne*, *Vaccinium*, and *Betula glandulosa* formed dense stands.

Saint-Lin Bog (45° 55′ N., 73° 47′ W.; alt. *ca.* 350 ft.) is situated on the southern ridge of the Canadian Shield, within, but close to, the northern limit of sea inundation. The kettle-type bog, about a quarter mile across, has a completely closed mat. The border consists of moraines or rocky ridges. Exploratory soundings indicated that the deepest part of the basin is in the central region. Here the boring was made. Considerable white pine still persists on the upland and the lower border has dense stands of red maple. Spruce, fir, tamarack, and birch constituted some of the neighboring forest cover

Lac des Plages Bog (46° 00′ N., 74° 51′ W.; alt. *ca.* 750 ft.) is a deep kettle, about 500 ft. across, bordered by high moraines and fringed by spruce, fir, pine, birch forest. The central part is a small open pool. It is located about one and a half miles east of Lac des Plages. Boring was made from a boat in the center of the pool. Since the pool is filled by about 10 ft. of *Fontinalis*, a second boring was made on the mat to include the latest forest history.

Lac à Pit Bog (46° 11′ N., 74° 29′ W.; alt. *ca.* 1000 ft.) is an almost circular beautiful deep kettle surrounded nearly completely by high moraines. The surface may be approximately 1000 ft. across. It too had a central open pool and so boring was made from a boat in the central part of the pool. Accumulation of *Fontinalis* interfered with sampling of upper layers, so a second boring on the mat is included as record of more recent forest history.

Mont Tremblant Bog (46° 15′ N., 74° 34′ W.; alt. *ca.* 2800 ft.) is a small kettle located at the base of Johannsen peak along the trail to the forestry tower. One end is still open water, but the lower, deeper portion is filled completely. The boring was made here. Surrounding forest is nearly all fir.

Lac Shaw Bog (46° 19′ N., 74° 32′ W.; alt. *ca.* 1200 ft.) is located west of Lac Shaw in the Mont Tremblant Park. The kettle is approximately 100 ft. across, but where the forest-covered portion of the basin is included it may be a quarter of a mile across. The steep wall of a granite ridge rises just a short distance from its north and northwest border, and the basin proper is surrounded by a low moraine. A forest of black spruce, fir, white cedar, and tamarack covers most of the older part of the mat.

Creek Savane Bog (46° 36′ N., 74° 12′ W.; alt. *ca.* 1300 ft.) is located in the eastern part of the Mont Tremblant Park, about six miles east from Lac Cyprès. The small oval kettle is bordered by sandy morainic uplands. Spruce is the predominating forest of the region but a recent fire has left the immediate neighborhood without forest cover.

Lac Rouge Bog (46° 56′ N., 74° 40′ W.; alt. below 1500 ft.), located close to the northern limit of the Mont Tremblant Park, is of the valley type surrounded by steep slopes of moraines 50 ft. high.

Mazanaskwa Bog (47° 07′ N., 74° 32′ W.; alt. below 1500 ft.). The deep-set oval kettle is about one fourth of a mile offshore from Lake Mazanaskwa. It is surrounded by extremely high moraines which are densely wooded with white pine, yellow and white birch, fir, and spruce. The center is a quaking mat, and no samples could be taken for a depth of 10 ft. A second boring nearer the bog border was made to obtain samples showing recent forest history. The lowest levels of peat were very dry and compacted, again suggesting an initial warm dry period and later paludification. The borer would not penetrate beyond the compacted organic layer.

Clova Bog (48° 07′ N., 75° 22′ W.; alt. *ca.* 1350 ft.) represents the twin kettle type, set in a sandy plain. Boring was made in the upper basin. A small stream flows from the lower basin. *Chamaedaphne* and *Ledum* form a

dense shrub association. Some black spruce and tamarack have established themselves on the mat. The surrounding forest is jack pine on sandy soil, black spruce, white birch, and aspen. We were told that no white pine was known within fifty miles southward of the station.

Lac Lacroix Bog (40° 02′ N., 75° 23′ W.; alt. *ca.* 1300 ft.) is a series of three small bog basins. The boring was made in the deepest of the basins.

Bachelor Lake Bog (49° 32′ N., 76° 08′ W.; alt. *ca.* 900 ft.). The circular basin bordered on a little stream, but was separated from it by a low moraine. The boring was made 500 ft. off the creek bank. The central open part of the bog is bordered by a forest of spruce, fir, birch. It is likely that jack pine was present on uplands. Alder formed dense stands near the stream.

Lac Soscumica Bog (50° 24′ N., 77° 46′ W.; alt. *ca.* 750 ft.) appears to have been a twin basin. One end of the dense sphagnum-covered mat borders on the lake. While a sandy low bar separates it from the lake, the peat appears to be cut by wave action which suggests that the lake expanded and encroached on the second basin (the bog). The boring was made about 200 ft. from the lake. Exploratory borings had shown this to be the deepest part. Casual observation indicated that the forest was chiefly black spruce with some tamarack on the mat. No fir was seen on the border.

Iroquois Falls Bog (50° 39′ N., 78° 02′ W.; alt. *ca.* 650 ft.). This too was a multiple basin arrangement. Numerous exploratory borings showed the deepest part to be in an open grass sedge meadow.

Horden Lake Bog (50° 54′ N., 77° 54′ W.; alt. below 500 ft.). The bog is located about 500 ft. from the sandy shore of Horden Lake. Originally it was a twin basin separated by a sandy wall. The upper, shallower end has a small open pool. Exploratory soundings disclosed the deepest part near the center of the basin. The boring was made here.

Smoky Hills Falls Bog (51° 27′ N., 78° 32′ W.; alt. *ca.* 175 ft.). The large bog is located 15 miles upstream from Rupert House on the Rupert River. It is about half a mile north from the river and separated from it by rocky outcrops and moraines. Ten exploratory borings disclosed little difference in depth of the basin. Gyttja-type organic material overlying without a transition the basal rock flour was collected by multiple-shots for carbon dating.

Rupert House Bog (51° 28′ N., 78° 45′ W.; alt. *ca.* 50 ft.). A muskeg-like open shallow basin one mile south of Rupert House. The vegetation is chiefly black spruce with some tamarack. No fir nor broadleaved trees were seen.

Jack River Bog (51° 59′ N., 78° 04′ W.; alt. below 500 ft.) is about 50 miles northeast of Rupert House. The depression is bordered by rocky outcrops, thus very likely constituting an ice scour basin. The rocky outcrops had pure stands of jack pine. Small black spruce and tamarack have invaded the mat in scattered colonies. The bog is located near the headwaters of the north branch of Jack River.

Observation

Climatic changes and influences of latitude are reflected in the forest history, and are at times difficult to separate. There was an initial warm period (Q-1) when jack pine, white pine, and oak (at its best representation) constituted the major forest cover northward to Clova, lat. 48° 07′ N. (Bog 9).

The initial warm period was soon followed by cooling climate (Q-2), which is reflected by a prominent increase of spruce and fir. This short cool period was replaced by the "major" warm–dry period (Q-3), when spruce and fir almost disappeared: at best they played a very insignificant part in the forest composition.

Jack pine had a period of major importance at all 19 stations, but from Clova to Jack River (Bogs 1–9) it had bimodal peaks. The low between the peaks resulted from expanding influence of white–red pine. The white–red pine element is very interesting in the part it played at various latitudes. It must be an indicator of warmer climate than that prevailing up north to-day. It penetrated as a significant component of the forest far beyond the present range, northward to James Bay (Bogs 1–9). Since then it has been depressed southward together with yellow birch while the most recent cooling (Q-5) of climate prevailed.

While, as stated, red–white pine extended to James Bay, its abundance was inversely proportional to increase in latitude, especially north of latitude 46° 19′ (Bog 13). It held an important position to recent times from Newington to Saint-Lin (Bogs 17–19). Microclimatic or edaphic factors enabled the larger pine to maintain significant abundance as far north as Lac à Pit and Lac Shaw (Bogs 13 and 15).

Two critical latitudes stand out prominently in the transect: Clova (48° 07′ N.) for coniferous species and paper birch, and the Lac Shaw region (46° 19′ N.) for a declining limit of *Tsuga*, *Fagus*, and *Quercus*. The latter two species never exerted dominating influence in the forest cover of Quebec, while *Tsuga* has a prominent peak in mid-profile from bogs of the Lac Shaw region. At Clova black spruce first shows great abundance, and associated with jack pine it replaced the initial white–red pine control. North of Horden Lake (Bog 4) succession is expressed very weakly. The great black spruce – jack pine belt develops into a single forest dominance, which impresses anyone flying over this belt in a plane. To-day this belt is replaced southward by a forest association of tamarack and black spruce. One wonders whether the black spruce – tamarack belt once occupied a more southerly position from that which it occupies to-day. It is unfortunate that the pollen record is unsatisfactory for tamarack. The sparse and disjunct representation of *Fagus*, *Quercus*, *Ulmus*, and *Tsuga* may merely indicate long distance pollen transfer and not actual invasion of the station areas. However, Halliday (9) lists white elm and black ash for his James Bay Section (B. 5).

High percentages of dwarf birch, or dwarf birch and alder, or dwarf birch, alder, and grasses, suggest tundra while deposition was in progress at Creek Savane (Bog 12), Mazanaskwa (Bog 10), Clova (Bog 9), Soscumica (Bog 6),

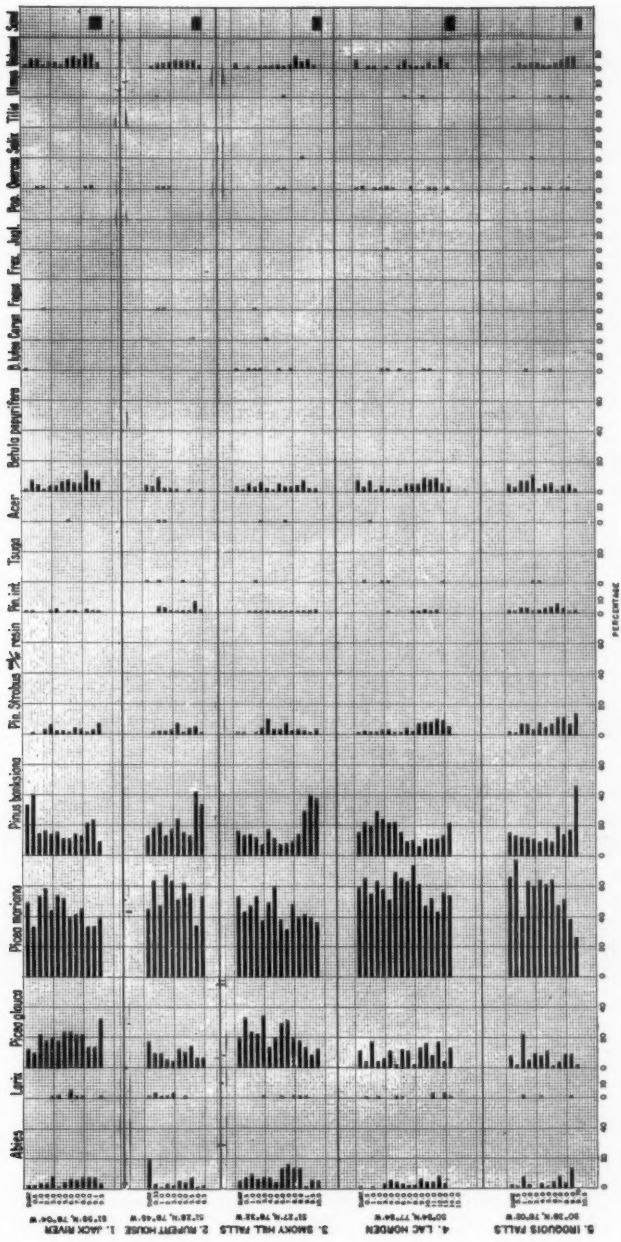

FIG. 4. Profiles of bogs 1, 2, 3, 4, 5.

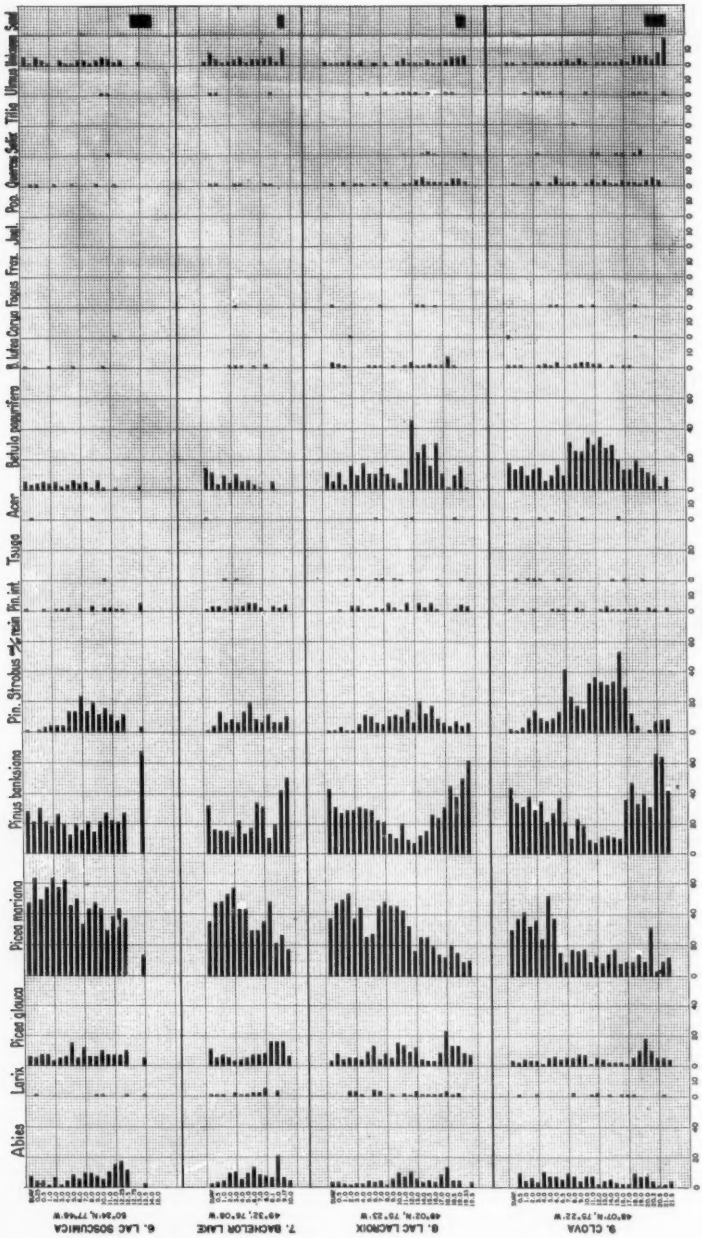

FIG. 5. Profiles of bogs 6, 7, 8, 9.

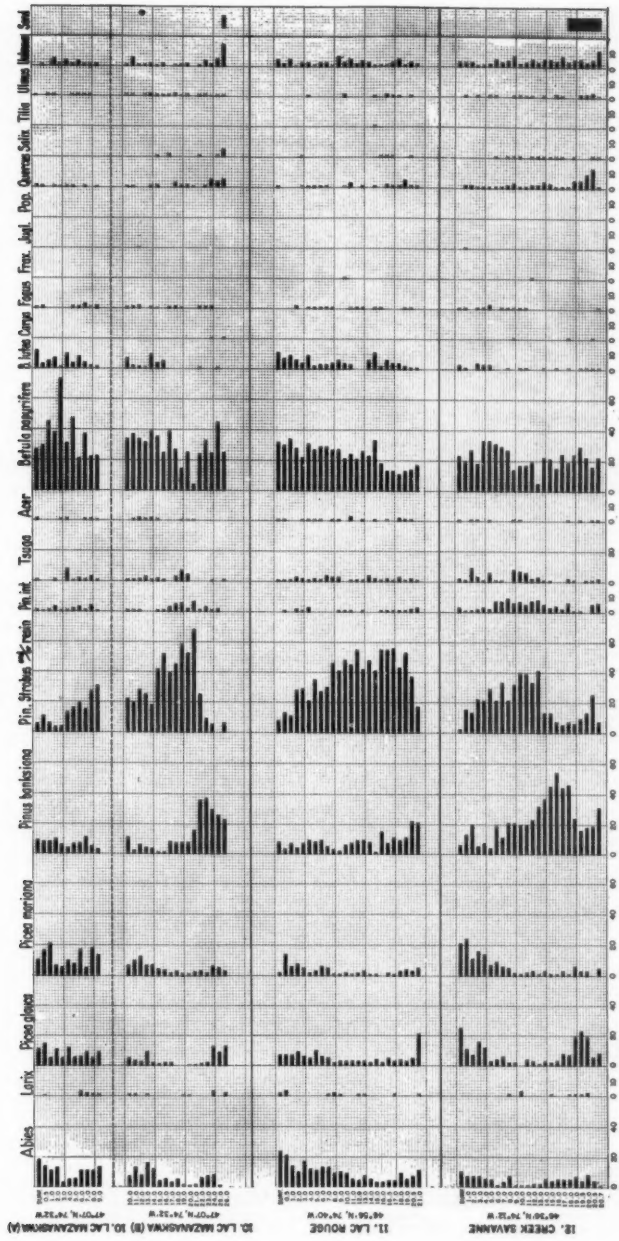

FIG. 6. Profiles of bogs 10, 11, 12.

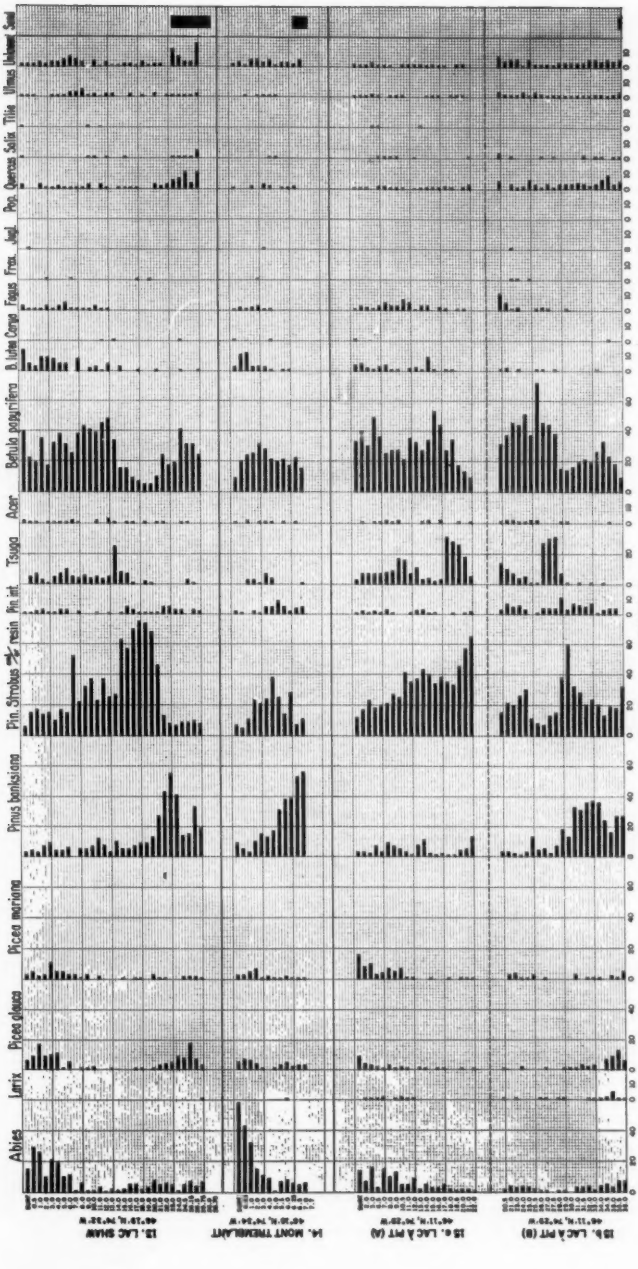

FIG. 7. Profiles of bogs 13, 14, 15.

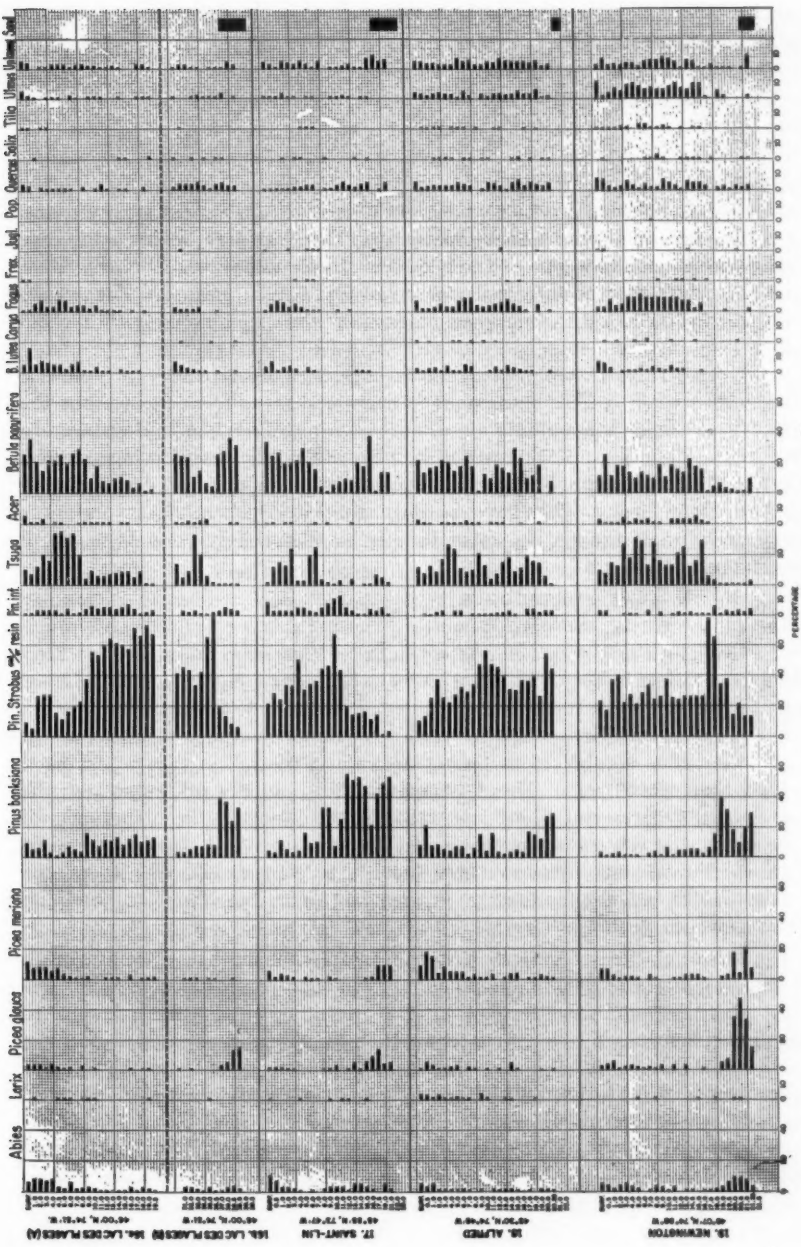

FIG. 8. Profiles of bogs 16, 17, 18, 19.

Smoky Hills Falls (Bog 3), and Jack River (Bog 1); in short, bogs which are mainly north of lat. 47° N. (Table I).

The Clova bog is outstanding in two respects, black spruce here first appears in great abundance and paper birch records a sharp decline northward of this location.

When one considers the highest percentage a species attains in the various latitudes (Fig. 2), the graph figure not only indicates temperature controls but also suggests the reason why certain species and genera appear early or later in pollen profiles. Most boreal in nature are *Picea mariana*, *Pinus banksiana*, *Picea glauca*, and to a lesser degree *Abies*. *Betula papyrifera*, *Pinus strobus*, and perhaps *Tsuga* are intermediate. *Quercus*, *Betula lutea*, and *Fagus* have least northward range. *Abies* presents some interesting characteristics which might be pointed out more specifically. It usually had its greatest abundance in the upper third of profiles, except in bogs between latitudes 49° and 51° 59′ N. (Bogs 1–8) where abundance is more or less uniform throughout the profiles. It reaches maximum abundance at Mont Tremblant (Bog 14). In the Mont Tremblant Park area it is apparently to-day replacing spruce. Potzger, Courtemanche, Sylvio, and Hueber (16) show this in a forest survey at Lac Shaw bog. This may indicate that in Quebec, *Abies* prefers a moderately cool climate; however it may express this preference in all of North America.

Discussion

Quebec is no doubt one of the most important areas in which to study postglacial forest history and forest changes in Eastern North America during post-Mankato time. It was one of the eastern centers of continental glaciation and so also a strategic location for records of minor fluctuations which might pass unrecorded southward of this center, where glaciation was transported rather than developed. The vast geographical northward extent of the Province also promises significant latitudinal differences in abundance of species which constituted the waves of succession in time. Such differences were evident already from southern Quebec to latitude 46° 30′ N. on the Laurentian Shield, as presented by Potzger and Courtemanche (13), involving especially such genera as *Quercus*, *Tsuga*, and *Ulmus*.

Centers of glaciation are also centers of climate which favors accumulation of glacial ice. It is thus quite evident that in such regions local glaciers are most likely to remain or develop without extending modifying influence to vegetation southward of such centers. Post-Mankato local glaciation in the Laurentide Park area has been established by the work of Osborne (11), and according to Flint (6) local glaciers persisted in widely separated areas of Eastern North America, of which the Shickshock Mountains, Gaspé Peninsula, might be referred to specifically. In a later publication Flint (7) suggests that the highlands of the Morin anorthosite (46° 30′ N., 74° 30′ W.) may have had local glaciers. The influence of local glaciation on forest succession in Quebec has been recognized by Potzger (12) and Potzger and Courtemanche

(13) as a regional feature, not only in the St. Lawrence valley but also on the Laurentian Shield. It stands out prominently in profiles from latitude 45° 07′ to latitude 50° 39′ N. in the present study (Figs. 5, 6, 7, 8). Flint (7) expresses the opinion that disappearance of the Laurentide ice sheet was complicated by the persistence of residual highland glaciers. This concept is well supported by the truncated forest history from Mont Tremblant bog (Bog 14) at the highest point of the region. Here forest history begins at the prominent jack pine period as it was being replaced by the white–red pine. At this period spruce–fir has declined to a negligible representation. It must be pointed out, however, that forest history not only suggests local glaciers, but also a general cooling soon after the advent of the major xerothermic period. During this time spruce–fir increased significantly, so that Potzger (12) and Potzger and Courtemanche (13) recognized it as a characteristic of pollen profiles from lower Quebec. In the present study the same characteristic was found in profiles of bogs northward to latitude 48° (Bogs 9 to 19). Local glaciers no doubt responded to this temporary cooling with expansion of area as Osborne (11) and Flint (7) point out.

The transect of bogs to James Bay would not have been possible without the aid of a plane, because of the inaccessible terrain beyond Clova. This method of field work is so ideal that one can hardly give adequate recognition to it. Four men can easily make a rapid exploratory survey to determine the nature of the basin and with division of duties involved in sampling can complete a boring in a short time in a most satisfactory manner. As stated by Potzger and Courtemanche (13) selection of bogs by plane is the best method in any rugged terrain even when such terrain is traversed by roads. Beyond the termini of roads this method becomes an absolute necessity for critical selection.

Results from a series of bogs are always more satisfactory than those from a single lake or bog, because regional patterns and variations due to edaphic or microclimatic differences cannot well, for lack of comparison, be read into a single profile. In a series of bogs, one profile supplements another, and in the present study influence of latitude is emphasized, affecting as it does the relative abundance of certain species in particular. Thus *Picea mariana* never expressed the dominance south of latitude 47° that it shows from Clova (lat. 48°) to Jack River (lat. 52°) (Figs. 4, 5).

Referring to another aspect of the study, there is further confirmation of the findings of Potzger and Wilson (17) with respect to work in bogs with central open pools. Borings made to one side of the pool in such incompletely closed bogs will usually be truncated and fail to record the earliest forest history. In the present study, unless borings were made in the pool itself, which necessitated the extra work of carrying a boat, the profiles were incomplete. We refer to borings A and B at Lac des Plages (Bog 16) and at Lac à Pit (Bog 15). In both instances the important initial warm period (Q-1) is not shown in borings at the border of the pool, and a slightly greater truncation would also miss the shorter cool period (local glaciation) (Q-2)

which is so characteristic for Quebec bogs. Auer (2) made a wrong conclusion about earliest forest composition and its climatic implications because he did not analyze the lowest sediments.

The long transect again emphasizes the point made by Potzger and Courtemanche (13) that the Mont Tremblant Park region is an important transition zone, especially for hemlock and various broadleaved genera such as *Fagus*, *Quercus*, *Betula*, and *Ulmus*, whose abundance tapers off between the St. Lawrence valley and the Mont Tremblant Park area. The fact that up to latitude 50° 39′ N. (Bog 5) the delayed initial spruce–fir peak makes its appearance suggests a rapid retreat of the ice cap and a quick advance of the forest. Since in southern latitudes of Quebec the short cold period (Q-2) was followed by rise in jack pine, we might assume that from latitude 47° 07′ N. (Bog 10) the major warm–dry period (Q-3) following the short earlier deterioration of climate marks the beginning of forest invasion. In regions of postglacial sea submergence, as in the James Bay region, inland to 600-ft. elevation, the close of the jack pine maximum and northward extension of white–red pine marks the beginning of forest history, which according to carbon dating reported by Potzger and Courtemanche (14) was between 2150 and 2550 years ago. It appears that this period also experienced increased precipitation resulting in development of expanding muskeg conditions, which in turn favored increase of black spruce.

In a private communication, D. M. Hopkins suggests care in the use of xerothermic period or climatic optimum, saying: "Evidence has accumulated since the advent of the C-14 method that the past 10,000 years have been punctuated by not one but several warm periods separated by notable glacial advance." One should no doubt be cautious with terminology but the shorter waves of warm periods do not always find expression in forest changes. South of the Great Lakes the progress indicates a single line increase in warmth to what appears and has been termed here "climatic optimum". This is no doubt what Hopkins (10) describes as "a single long post-Mankato warm interval that extended from about 9,000 to about 3,500 years ago". Here this author describes a condition in Alaska, but the same change is noticeable in Eastern North America. Potzger (12) and Potzger and Courtemanche (13) expressed the opinion that this major and apparently also extreme xerothermic period is responsible for rapid melting of the Laurentide ice cap.

In Quebec this major xerothermic period was preceded by a pronounced cooling which rejuvenated local glaciers (if they had persisted as residual highland glaciers) or initiated them. Following this interruption there was a long, apparently uninterrupted warm period, warmer than during any time of the past 3000 years, during which time spruce and fir had disappeared almost completely from the St. Lawrence northward to latitude 47° 07′ N., 74° 32′ W. (Figs. 6, 7, 8).

If upwarping of the land adjacent to Hudson Bay was due to wasting ice in northern Quebec, then the long warm period must have persisted to approximately 3000 years ago, when marine waters withdrew from the region around

Hudson Bay and permitted forests to become established, as Potzger and Courtemanche (14) found at the Smoky Hills Falls bog.

It is difficult to say if latitude alone or if delayed wastage of ice operated in determining a forest history in which spruce played a major role from the beginning. This is characteristic of profiles beginning about 48° N. (Bog 9). One is no doubt justified in assuming that forests invaded this latitude at the close of the major warm period from the fact that oak pollen has greatest abundance in the lowest levels (Bogs 8–12). Marine submergence may veil the climatic controls since trees could not invade areas below 600-ft. elevation until withdrawal of marine waters.

If one plots the maxima of percentages for the various genera of trees as a graph (Fig. 2) one derives significant facts on distribution as well as on climatic controls, but besides the mere fact of northern or southern preference of the various species and genera, the region about Clova and the region of the Cochrane moraine stand out as critical migration points for *Picea mariana*, *Betula papyrifera*, and *Pinus strobus*. The Mont Tremblant region marks a critical latitude for *Tsuga*, *Betula lutea*, and *Fagus*. The usual low abundance of *Abies* makes the customary term "spruce–fir" for the boreal forest somewhat doubtful. In reality, *Abies* appears to be more subboreal in its preference. *Fagus* and *Betula lutea* are the outstanding most recent invaders with limits at or south of Clova. All indications are that areas north of Clova were invaded by forest close to the waning of the major xerothemic period (Figs. 4, 5) because here the initial warm period and shorter cold period are absent while white pine shows prominence at the very beginning. We might also say that vegetational history began up there during the white pine – hemlock – beech – white birch phase south of lat. 47° (Mazanaskwa bog), and only white pine had the potential adaptability to advance northward to James Bay (lat. 51° 24′). It is therefore somewhat difficult to correlate forest type with time without involving climatic differences due to latitude at any given time. However, it appears to be quite definite that areas north of lat. 48° (Clova) were invaded by forests late during the xerothermic period (Q-3) or shortly before the recent cooling (Q-5) when spruce and fir together with yellow birch replaced pine in the Mont Tremblant Park region. Why jack pine did not make significant reinvasion of the region south of Clova during the recent cool period is difficult to explain. Theoretically one would expect it. Such a reinvasion is, however, weakly expressed at Creek Savane (Bog 12) and Lake Mazanaskwa (Bog 10).

The "old warhorse" of American palynologists, the tundra, enters into the Quebec profiles as a problem not definitely solved. Workers have not united opinion on what constitutes tundra evidence. Deevey (3) leans heavily on grass–sedge pollens. From personal observations in tundra regions of northern Quebec the writers know that dwarf willow, dwarf birches, and various species of ericads characterize the tundra, while grasses and sedges may in some places be considered important species in the sparse vegetation cover. Also, Potzger and Courtemanche (manuscript) found pollens of these shrubs poorly repre-

sented in moss polsters of the region. Based on abundance of pollens of *Betula glandulosa*, *Alnus*, grasses, and sedges, tundra might be assumed at six stations, namely, Bogs 1, 3, 6, 9, 10, and 12.

Beyond lat. 49° N. the line of bogs cut across three important physiographic features, i.e. the glacial lake Barlow-Ojibway, the Cochrane moraine, and the marine submergence. It is quite obvious that areas of the glacial lake and the marine submergence could not have been invaded by forests until the waters receded. The carbon dating reported by Potzger and Courtemanche (14) from the Smoky Hills Falls bog suggests 2350 ± 200 years ago when forests could enter the marine invaded area.

The question now arises: where does the Cochrane substage fit into the forest history? Two points might be considered. It could be read into the shorter spruce–fir period (Q-2) following the initial shorter warm period (Q-1), which appears northward to lat 46° 36′ N. (Creek Savane), or it might be represented by the spruce–fir peak in upper sections of profiles so evident in bogs up to lat. 48° N. The most likely place to look for the Cochrane stage is the shorter spruce–fir period (Q-2), following the initial short warm period (Q-1). Antevs (in private communication) reads it into this point of the Quebec profiles.

If one attempts to correlate forest history with such geological pivot points as Timiskaming retreat, Cochrane stage, which marked a dying glacial age, but also decline of Lake Algonquin, Champlain Sea, and Ottawa Sea, the transect of 19 bogs yields some very significant correlations between forest history and geologic phenomena. Antevs (1) considers the Cochrane halt as part of late Lake Timiskaming age which he considers correlative with the final drainage of Lake Algonquin and regression of the Champlain Sea. According to Potzger (12) the southern Quebec forest history suggests that this occurred during the initial short warm period (Q-1), and Potzger and Courtemanche (15) find this indicated in a series of bogs in the Gatineau valley in the strategic area about Maniwaki and Kazabazua. These profiles clearly suggest the Ottawa valley as early migration route for southern broadleaved species. We are perhaps justified in assuming that Newington bog (Bog 19) began at the close of the Champlain Sea and thus records all of post-Cochrane time, or perhaps from close of the early warm period (Q-1), late Timiskaming, to the present.

The Alfred bog (Bog 18) indicates truncation, but in a private communication Antevs has the following interesting explanation: "Site 18 (Alfred Bog) appears to have a critical altitude. If it stands less than 225 feet above the sea level, it may have been flooded by the Ottawa marine stage; and then the whole bog may be post-Cochrane." One thing is certain, the forest history is comparable to that beginning at the 19-ft. level of the Newington bog (total depth 21 ft. 8 in.), marking the beginning of the major warm period (Q-3), and so is lacking climatic changes Q-1 and Q-2. Submergence by the Ottawa Sea or truncation due to the sampling in an off-center location are possible explanations for the incomplete profile of the Alfred bog. The latter is not unlikely in so expansive an area, but the former would be more significant.

Clova (Bog 9) was previously referred to as a critical location. If according to Wilson (18) the Cochrane moraine stood at about 50° N., 78° W. then Clova is in an important location with reference to the Cochrane moraine. This suggests the probability that peat began to accumulate there soon after the ice melted back, and that Lac Lacroix bog began to form shortly after (Bog 8). Western Quebec thus presents one of the finest opportunities to associate pollen profiles with known geologic happenings, as well as with latitudinal selection of forest dominants.

Correlation of initial forest history recorded in bogs at various latitudes with Timiskaming retreat and Cochrane halt glacial history is as follows:

Recent: Rupert House

Late post-Cochrane to Recent: Mont Tremblant, Lac Horden, Iroquois Falls, Lac Soscumica, Smoky Hills Falls, Jack River

Early post-Cochrane: Alfred, Lac Lacroix, Bachelor Lake

Cochrane halt: Lac des Plages (?), Lac Rouge, Lac Mazanaskwa, Clova

Timiskaming retreat: Newington, Saint-Lin, Lac à Pit, Lac Shaw, Creek Savane

If one correlates lowermost levels of bogs north of 48° with the Clova bog profile (depth 21.5 ft.) successive later initiation of sedimentation in bogs northward is indicated as follows: Lac Lacroix bog, comparable to Clova 20-ft. level; Bachelor Lake bog and Iroquois Falls bog, to 10-ft. level; Horden Lake bog and Lac Soscumica bog, to 6-ft. level; Smoky Hills Falls bog and Jack River, to 4-ft. level; Rupert House bog, to 3-ft. level or above.

The James Bay transect is most gratifying in its correlations on the one hand with previous analyses in lower Quebec by Potzger (12) and the extensive study by Potzger and Courtemanche (13) at the Mont Tremblant Park in the Laurentians, and on the other hand with interpretations by geologists of late glacial happenings, based purely on physical phenomena. This points to desirable more frequent coordination of research by the two groups of scientists.

Wide generalizations are withheld until collections made on the 1954 plane trip from the James Bay region into the tundra and the collections of the 1955 plane trip in the Cochrane moraine area have been analyzed.

References

1. Antevs, E. Geochronology of the deglacial and neothermal ages. J. Geol. 61 : 195-230. 1953.
2. Auer, V. Peat bogs in southeastern Canada. Geol. Survey Can. Mem. 162. 1930.
3. Deevey, E. S. Late glacial and post-glacial pollen diagrams from Maine. Am. J. Sci. 249 : 177-207. 1951.
4. Dresser, J. A. and Denis, T. G. Geology of Quebec. Vol II. Geol. Rept. 20, Dept. of Mines, Quebec. 1944.
5. Erdtman, G. An introduction to pollen analysis. Chronica Botanica Company, Waltham, Mass. 1943.
6. Flint, R. F. Glacial geology and the pleistocene epoch. John Wiley & Sons, Inc., New York. 1948.

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7. FLINT, R. F. Highland centers of former glacial outflow in northeastern North America. Bull. Geol. Soc. Amer. 62 : 21-38. 1951.

8. GEISLER, F. A new method in separating fossil pollen from peat. Butler Univ. Botan. Studies, 3 : 141-149. 1935.

9. HALLIDAY, W. E. D. A forest classification for Canada. Can. Dept. Mines, Resources, Lands, Parks. Forests Branch, Dominion Forest Service. Bull. 89. 1937.

10. HOPKINS, D. M. and GIDDINGS, J. L., JR. Geological background of the Iyatayet archeological site, Cape Denbigh, Alaska. Smithsonian Misc. Collections, 121, No. 11. 1953.

11. OSBORNE, F. F. Parc des Laurentides ice cap and the Quebec Sea. Naturaliste can. 78 : 221-251. 1951.

12. POTZGER, J. E. Nineteen bogs from southern Quebec. Can. J. Botany, 31 : 383-401. 1953; Bull. Serv. biogéograph. 9. 1953.

13. POTZGER, J. E. and COURTEMANCHE, A. Bog and lake studies on the Laurentian Shield in Mont Tremblant Park, Quebec. Can. J. Botany, 32 : 549-560. 1954; Bull. Serv. biogéograph. 11. 1954.

14. POTZGER, J. E. and COURTEMANCHE, A. A radiocarbon dating of peat from James Bay in Quebec. Science, 119 : 908-909. 1954.

15. POTZGER, J. E. and COURTEMANCHE, A. Five bogs from the Gatineau valley, Quebec. Butler Univ. Botan. Studies, 1956. In press.

16. POTZGER, J. E., COURTEMANCHE, A., SYLVIO, M., f.e.c., and HUEBER, F. Pollen from moss polsters on the mat of Lac Shaw bog, Quebec. Correlated with a forest survey. Butler Univ. Botan. Studies, 1956. In press.

17. POTZGER, J. E. and WILSON, I. T. A study on lake filling and bog formation. Proc. Indiana Acad. Sci. 54 : 78-80. 1945.

18. WILSON, J. T. Glacial geology of part of northwestern Quebec. Trans. Roy. Soc. Can. IV : 49-59. 1938.

CHROMOSOMES AND TAXONOMY OF EASTERN NORTH AMERICAN POLYGONUM[1]

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Abstract

It is pointed out that cytological, palynological, and morphological investigations support the division of the collective genus *Polygonum* into natural units. The 13 sections of the collective genus are grouped into six natural genera, two of which include five and four sections, respectively, while the remaining four genera are monosectional. The present paper is a report on combined cytological and taxonomical studies of the genus *Polygonum* s.str. in eastern North America, including 25 taxa of the section *Polygonum* (= *Aviculatia*) and one species of the section *Duravia*. The basic number of chromosomes of *Polygonum* s.str. is $x = 10$; all the chromosomes have an almost median centromere and thus cannot be morphologically distinguished from each other. The chromosome numbers within the group range from the diploid number $2n = 20$ to the octoploid number $2n = 80$. Nine species (12 taxa) are diploid; seven species (eight taxa) are tetraploid; four species (five taxa) are hexaploid, and one species is octoploid. Hybrids between several of these taxa revealed that while most species with even the same chromosome number are so different cytogenetically that their offspring are almost completely sterile, others show such close relationship that they cannot be regarded as more distinct than are intraspecific races. In spite of the use here of a system very different from those accepted in recent North American manuals, no new names or combinations are needed, since all the taxa here studied have been described in acceptable categories by previous botanists. It has been suggested that the great variability of at least one of the collective species included in recent North American manuals might be due to the occurrence of apomixis. This suggestion could not be verified, although a low degree of facultative apomixis is possible. The variation which occurs in the same locality is shown to be caused mainly by the fact that the false populations are composed of several morphologically and biologically distinct taxa at the species level, and the constancy of intraspecific races, even when they are growing together as a result of the activities of man, seems to be the result of a high degree of autogamy.

I. Introduction

The genus *Polygonum*, in the collective sense of many recent manuals, contains some of the most widespread and common plants on the North American continent. Its species are as frequent in dry and sandy soils as they are in marshes and cultivated fields, and they occur throughout the subtropical, temperate, boreal, and subarctic regions. Some taxa are strictly maritime, while others are exclusively montane; many evince no particular edaphic or climatic preferences. There are species reaching up to 4 meters in height and others rarely surpassing 1 centimeter, and the variation in morphological characters of all categories is so great that the reasons for their allocation to only one genus are difficult to comprehend.

In the past the collective genus *Polygonum* has been repeatedly split into smaller and more natural units solely on the basis of morphological differences. Meisner (50, 51) and many others (cf. 2, 23, 78) named these units as equivalent sections, while several scientists (1, 7, 20, 21, 22, 23, 53, 54, 55,

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63, 64, 68, 69, 70, 71, 72, 73, 74, 75) regarded them as equivalent genera. The former treatment is followed in recent manuals by Marie-Victorin (49), Komarov (30), Fernald (17), Gleason (19), Clapham, Tutin, and Warburg (10), Hultén (25) (though treating the sections as subgenera), and Hylander (27). Palynological studies by Hedberg (23), physiological studies by Jaretzky (28), and cytological investigations mainly by Jaretzky (29), but also by Löve and Löve (44) and others, had shown that an intermediate scheme of classification must be adopted if the taxonomy of this group is to be based on common origin and evolutionary principles. In such a system of natural classification some of the sections by Meisner (51) and others are to be retained as such, while others undoubtedly belong to distinct genera which must differ considerably from each other in phylogeny. Thus, the 13 sections usually included in *Polygonum* s. lat. are divided into six genera, which are not only morphologically and palynologically clearly distinct (23) but also cytologically well separated by both different basic numbers of chromosomes and differences in chromosome size and form at mitosis. The chromosomes of taxa separated as genera are also so distinct genetically that natural hybrids are unknown and all hybridization experiments have been unsuccessful. However, within each genus the sections are more closely related in morphology, and in addition to having the same basic number of chromosomes, they show sufficient genetic relationship to allow some low degree of hybridization, although the rarely obtained hybrids of homoploid parents are highly sterile. Some details of these investigations have been mentioned by Löve and Löve (47) and further particulars will be published on a later occasion. The taxa regarded as separate genera on the basis of these studies are as distinct in morphology and cytology as are other equivalent and long accepted groups of the family, and they are more disparate in their evolution than the subgenera of *Rumex* (cf. 58, 59, 60) which are sometimes regarded as good genera (cf. 41, 44, 48).

The natural genera split out of the collective genus *Polygonum* are not equally large, and only two of them include more than one section. The former sections *Bistorta*, *Aconogonon*, *Tiniaria*, and *Pleuropterus* form the genera *Bistorta* Mill., *Pleuropteropyrum* Gross, *Bilderdykia* Dum., and *Reynoutria* Houtt. respectively. The five sections *Persicaria*, *Amblyogonum*, *Cephalophilon*, *Echinocaulon*, and *Tovara* are retained as equivalent sections of the genus *Persicaria* Mill. The last-mentioned section is regarded as a separate genus by Fernald (17) on the basis of its morphological distinctiveness, while Gleason (19) using the same criteria lists it only as a section, though with the remark that of all the sections of *Polygonum* s.lat. *Tovara* "has the best claim to generic recognition." The treatment of it as a section and not as a genus is supported by cytogenetical and palynological evidences. However, it should be pointed out that from a cytogenetical viewpoint *Tovara* differs more widely from the other four sections of *Persicaria* than these sections do from each other; thus, if an evolutionary distinction were made to indicate the observed differences, *Persicaria* could be divided into two subgenera, one of which should

include only *Tovara*. Nevertheless, these sections are more closely related to each other than they are to the other genera. Cytological as well as morphological and palynological observations support the whole scheme of classification outlined above, with the sole exception that palynological data (23) and some morphological similarities may seem to indicate a generic association of *Bilderdykia* and *Reynoutria*, while this is strongly contradicted by the available cytological data. However, it is very likely that if a few species, now tentatively grouped with the latter genus, are transferred to the former, this discrepancy will also disappear. The remaining four sections of the collective group—*Polygonum* (=*Avicularia*), *Duravia*, *Pseudomollia*, and *Tephis*—are again closely related to each other but clearly distinct in morphology, palynology, and, in particular, cytology from all the other groups, and they are doubtlessly congeneric. Since *Polygonum aviculare* L. is the type species of the genus *Polygonum* (9), the generic name *Polygonum* is to be retained for this last genus according to the International Code.

The present paper is designed as the first contribution towards the understanding of the cytogenetical and taxonomical variability within and between the different genera and sections mentioned. It comprises only the eastern North American species of the genus *Polygonum* in its strict sense, and since only one species of this group in our region—*Polygonum Douglasii*—belongs to the predominantly Pacific section *Duravia* S. Wats., almost all the observations reported here are confined to the section *Polygonum* itself. This is probably the most confused group within the family, as shown among others by the fact that the two most recent North American manuals, by Fernald (17) and Gleason (19), accept 14 and 11 native and naturalized species respectively, while the present treatment shows that at least 21 equivalent species and 26 morphologically distinct taxa occur in the region covered by these manuals. All these taxa have been described in their respective categories by previous taxonomists, but their acceptance by authors of manuals has been delayed mainly because of lack of authentic material or because the non-specialist of the genus was unable to classify this enormous variation as easily as the taxa of other less variable groups.

The cytological and taxonomical material studied by the present writers has been collected mainly in localities from the Atlantic coast west to Manitoba or, in some few cases, has been cultivated from seeds from recent herbarium sheets. Voucher specimens are kept in the Herbarium of the University of Manitoba. The cytological methods used were those described in detail by Löve and Löve (46) with the modifications mentioned by Löve and Sarkar (48).

II. Cytological Observations

A. Previous Reports

The first cytological investigations on species of the genus *Polygonum* in its strict sense were made by Jaretzky (29). His material seems to have originated mainly from seeds from Botanical Gardens rather than from natural habitats in or near Kiel in Schleswig-Holstein, and it included only six taxa.

The diploid chromosome number $2n = 20$ were reported for the species *P. Bellardi* (= *P. patulum* M.B.) and *P. maritimum* L., while the tetraploid number $2n = 40$ was counted for *P. argyrocoleon* Steud., *P. aviculare* L., *P. monspeliense* Thieb., and *P. plebejum* R. Br. The next report was that by Löve and Löve (42) giving the tetraploid number $2n = 40$ for Swedish material of "*P. aviculare*" with the remark that the specimens studied seemed to be closely related to *P. heterophyllum* Lindm. This identification was, however, erroneous, since the voucher specimens belong to *P. neglectum* Bess., which at that time was unknown to the writers since it is not mentioned in Scandinavian floras, except as variety *angustissimum* (Meisn.) Lindm. of *P. heterophyllum* in the Swedish flora by Lindman (36). The same year Andersson (in (43)) published the numbers $2n = 40$ and 60 for Swedish specimens of *P. aviculare* s.lat., while later (in (44)) he reported the former number for *P. aequale* Lindm. (= *P. aviculare* s.str.) and the latter (and occasionally $2n = 50$) for *P. heterophyllum* Lindm. Pólya (57) studied Hungarian material of *P. aviculare* and confirmed the tetraploid number for them. Finally, Löve (40) and Löve and Löve (47) found that Icelandic material of *P. aviculare* ssp. *aviculare* and ssp. *calcatum* (Lindm.) Thell. has $2n = 40$ chromosomes, while plants of *P. heterophyllum* ssp. *heterophyllum* and ssp. *boreale* (Lge) Löve and Löve have $2n = 60$ chromosomes.

B. The Eastern North American Taxa

1. Polygonum Douglasii Greene

A plant of rocky and dry soils and with a boreal distribution, this species is characterized by its leaf form, the rather large fruiting calyx, and the long, weak stalk of the flower which results in an early recurvation of the fruit, a character which is absent from the other eastern American species. Plants from different localities on the prairies were found to be tetraploid with $2n = 40$ chromosomes (Fig. 1).

This is the only taxon of the section *Duravia* which reaches the eastern parts of the continent, all other species of that group being typical of the west coast or the Rockies. Although Rydberg (64) felt that it might be introduced east of the Rockies, the present writers doubt the correctness of that suggestion at least for a substantial part of the area occupied by the species in the northeastern parts of the area.

Hybrids between this species and the other eastern taxa are unknown in nature and very difficult to obtain experimentally. Viable seeds giving weak seedlings which soon perish have been obtained from the cross between it and *P. tenue*, which lies closer to section *Duravia* than any other taxon here grouped in section *Polygonum;* and inviable seeds were also obtained from pollinations with *P. ramosissimum*, but so far not from other species of this section.

2. Polygonum tenue Michx.

A southern plant of dry soils, this species is characterized by its rather long and ascending stems with narrowly lanceolate leaves and the flowers in the axils of much reduced leaves. Only one collection from northern Minnesota

was studied. The chromosome number was found to be $2n = 20$ (Fig. 2), and that number was also counted on material grown from seeds of the very local var. *protrusum* Fern. (16, 17) from southeastern Virginia (Fig. 3). The variety differs from the typical race mainly in the much shorter calyx at the fruiting stage, so that the achene is exserted from the calyx instead of being enclosed by it. This character is also met within other taxa and is usually taxonomically rather significant and may even indicate specific differences. Since fully fertile hybrids with normal meiotic divisions are easily produced between var. *tenue* and var. *protrusum*, and since the distinguishing character shows a simple dominance of the longer calyx in F_1 and a clear 3 : 1 ratio in F_2, it is clear that this race is not more than a variety.

3. Polygonum arenarium W. & K.

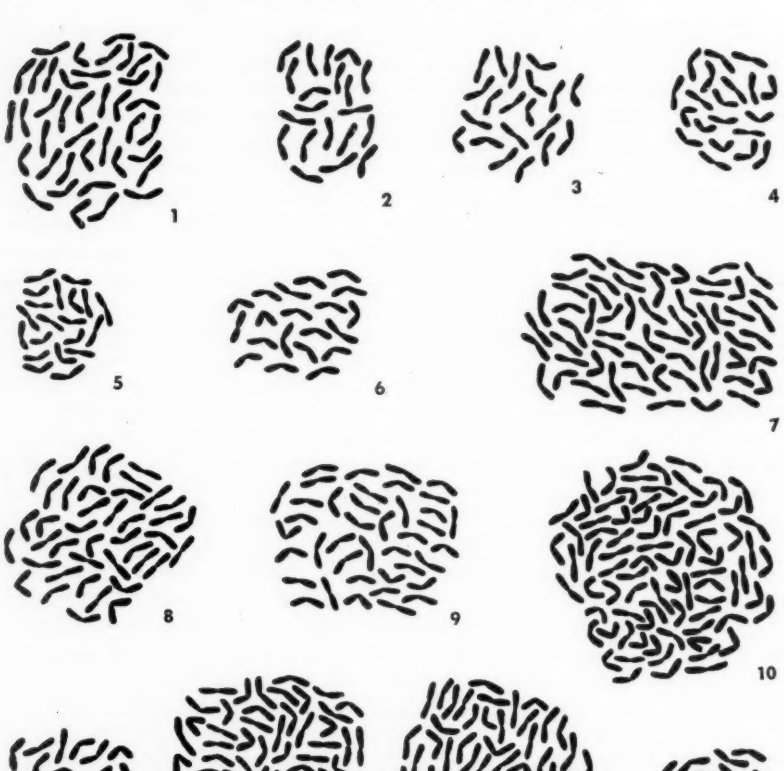
This eastern European and western Asiatic species with tall and diffuse stems has naked raceme because of the deciduous character of the upper leaves. It is a weed established only in some harbor and railway station areas in the eastern parts of the continent and near some farmlands in the west. It is a diploid with $2n = 20$ chromosomes (Fig. 4).

4. Polygonum patulum M. B.

A native of Eurasia, this species is occasionally adventive in waste places and near warehouses in eastern North America at least as far west as Manitoba. It is the taxon listed as *P. Bellardi* All. by Gleason (19), but since the original description of that species indicates a plant very similar to *P. rurivagum* and is certainly not synonymous with *P. patulum*, the name *P. Bellardi* is to be dropped as a *nomen confusum*. The typical ssp. *patulum* is a rather rare race in North America; it is diploid with $2n = 20$ chromosomes (Fig. 5) as already demonstrated by Jaretzky (29). However, the authors have mainly collected American material of the ssp. *Kitaibelianum* (Sádl.) Jáv. (cf. 76), which sometimes is regarded as a distinct species (27). Its chromosome number is also $2n = 20$ (Fig. 6), and since hybrids between both these races are not infrequent and at least almost fully fertile showing only a low degree of meiotic disturbance, this must be taken as additional support for the classification of both only as races of the same species.

5. Polygonum Raii Bab.

Originally described from the British Isles, this is a species of sandy shores and fine coastal shingle. Its achene is exserted and is similar to that of *P. oxyspermum* and *P. glaucum*, but the plant is more robust, with leaves ranging from elliptic-lanceolate to linear-lanceolate, and with a rather long ochrea which often is distinctly veined. The species is met with in Europe on the coasts from northern Scandinavia south to northern Spain, while reports from the Mediterranean (cf. 10) most probably are based on the closely related *P. Roberti* Lois., and those from between Denmark and the Channel are something else. In North America the species seems to be confined to the coasts of Newfoundland, the Magdalen Islands, and northeastern New



FIGS. 1–14. Mitotic chromosomes of American *Polygonum*. Magnification × *ca.* 3500. FIG. 1. *P. Douglasii*, $2n = 40$. FIG. 2. *P. tenue* var. *tenue*, $2n = 20$. FIG. 3. *P. tenue* var. *protrusum*, $2n = 20$. FIG. 4. *P. arenarium*, $2n = 20$. FIG. 5. *P. patulum* ssp. *patulum*, $2n = 20$. FIG. 6. *P. patulum* ssp. *Kitaibelianum*, $2n = 20$. FIG. 7. *P. Raii*, $2n = 60$. FIG. 8. *P. glaucum*, $2n = 40$. FIG. 9. *P. Fowleri*, $2n = 40$. FIG. 10. *P. oxyspermum*, $2n = 80$. FIG. 11. *P. autumnale*, $2n = 20$. FIG. 12. *P. exsertum*, $2n = 60$. FIG. 13. *P. allocarpum*, $2n = 60$. FIG. 14. *P. proliferum*, $2n = 20$.

Brunswick south to southwestern Nova Scotia (cf. Fig. 15). Since there is no doubt as to the indigenous nature of the American populations, the inclusion of the species in the group of European seashore plants by Hultén (26) must have been based on some misunderstanding. More correctly it can be said to be an Atlantic species with its main area in Europe and a few outposts in eastern North America, or a boreal eastern amphi-Atlantic plant. It is absent from the Faeroes, Iceland, and Greenland.

It is known (65) that at least two morphologically distinct races of the species occur in Europe, namely the typical race in the British Isles and probably further south, and a more northern, or Scandinavian, race having a more blue-green foliage, shorter internodes, flowers which are white rather than pink or greenish-white, and somewhat broader achenes. The Scandinavian race is named ssp. *norvegicum* Sam. The American plants seen by the writers seem to be more closely related to the latter than to the British specimens, an observation which certainly supports the view that it is truly indigenous here, since if introduced it would hardly be more nearly related to populations from Scandinavia. The American populations are considerably less variable than are the European races, but although they are probably separable even from the Scandinavian ones, the lack of distinct technical characters makes their taxonomical separation impracticable.

The chromosome number has not yet been determined for European material of *P. Raii*, but plants grown from seeds from Nova Scotia were found to be hexaploid with $2n = 60$ chromosomes (Fig. 7).

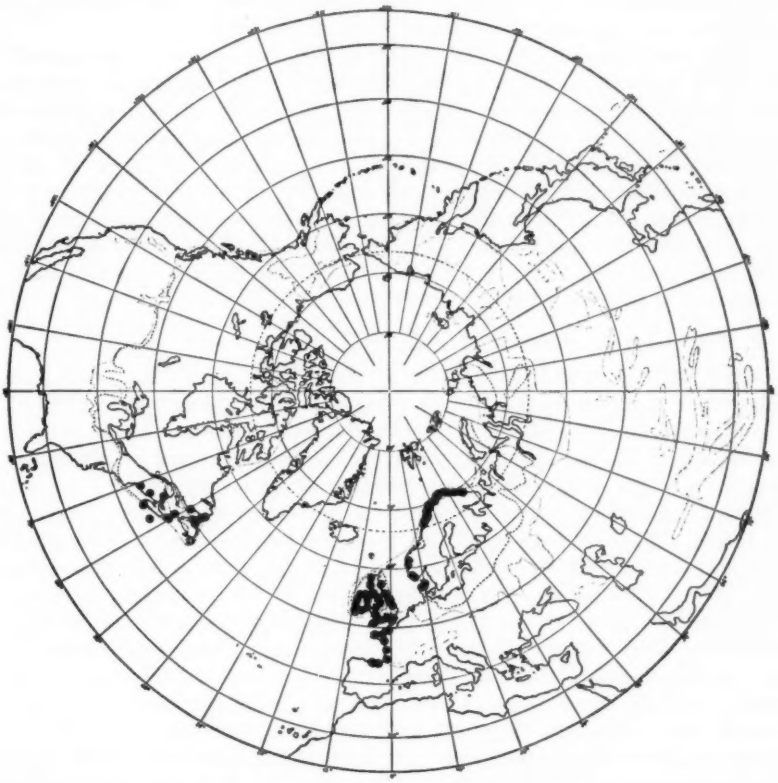


FIG. 15. Distribution area of *Polygonum Raii*.

6. Polygonum glaucum Nutt.

This American plant of saline and sandy shores of the more temperate Atlantic coast (cf. 56) is probably related to the Old World species *P. maritimum* L. (cf. 12, 13, 14, 17) and specimens from Virginia were actually wrongly identified with that taxon by Linnaeus (37) himself, and by several later authors. It differs from the European plants in characters of the achene and flowers, and also in the leaf-form and ochrea. Also, it differs from it in its chromosome number, since the American plant is a tetraploid with $2n = 40$ chromosomes (Fig. 8) while the European species is a diploid (29, 61*a*). However, the American taxon is hardly an autotetraploid of the European one, since the total lack of multivalents at meiosis, despite a rather high frequency of chiasmata caused by the formation of mainly ring-bivalents, is probably a good indication of an alloploid derivation.

7. Polygonum Fowleri Robins.

This species is said to be endemic on sandy or gravelly shores in the northeast, from southeastern Labrador and Newfoundland south to lower St. Lawrence and the eastern coasts of Nova Scotia (15, 17, 62, 67). It is, however, also native on the Pacific coast (cf. 25), from where it was originally described by Nuttall (in (5)) under the homonym *P. buxifolium;* it is also met with in easternmost Asia (30). Its total area is approximately indicated in Fig. 16. Meisner (51) listed this taxon as a variety *buxifolium* of *P. littorale* Link, while Ledebour (32) transferred it as a variety of *P. aviculare.* The plant has a fleshy stem and more succulent leaves than other American species of the genus, a rather long ochrea with inconspicuous veins, and the achene broadly ovoid and clearly exserted. The chromosome number of specimens cultivated from seeds from herbarium material from the east and west coasts was found to be $2n = 40$ (Fig. 9).

8. Polygonum oxyspermum Mey. & Bge.

When first discovered by Fernald (13) from Nova Scotia, this species was thought to be an eastern American endemic. It was named *P. acadiense* Fern. until Samuelsson (65) identified it with the south Scandinavian and Baltic species described from the sandy shores of the island of Oesel by Meyer and Bunge (in (31)). Although it is morphologically rather distinct, it is perhaps best characterized by its very long and exserted olivaceous achenes. According to Hultén (26) it belongs to the group of incompletely boreal-circumpolar plants with gaps in eastern Asia and in America. Since the gap reaches all the way from the Atlantic coasts of Nova Scotia, which is the only American station for the species (cf. 13, 14, 17, 62), westwards through America, Siberia, and Russia to the shores of the Baltic countries and southern Scandinavia (cf. 26, 30), which are its only localities in Europe, it is probably more appropriate and less confusing to regard it as a boreal amphi-Atlantic plant (cf. Fig. 17). European material has not yet been studied cytologically, but specimens grown from seeds from the Bras-d'Or Lakes were found to be octoploid with $2n = 80$ chromosomes (Fig. 10).

FIG. 16. Distribution area of *Polygonum Fowleri*.

9. Polygonum autumnale Brenckle

Described recently by Brenckle (8), this species has not been accepted by Fernald (17), Gleason (19), and other authors of recent manuals, except Stevens (77), although it seems to be rather frequent at least in the southern part of the area covered by these floras. It is a roadside plant, uncommon in southern Manitoba, with high and slender stems bearing linear to lanceolate leaves, and flattened, three-angled, reddish-brown achenes. The ochreae of young stems and branches are large and shining white. Although the plant resembles *P. aviculare* in general habit and type of fruit, and *P. neglectum* or even *P. ramosissimum* in the form of branching and leaves, especially at midsummer when most of the leaves have fallen off, it cannot be a hybrid between any of these species, not only because of its full fertility but mainly because of its chromosome number. It is a diploid plant with $2n = 20$ chromosomes (Fig. 11).

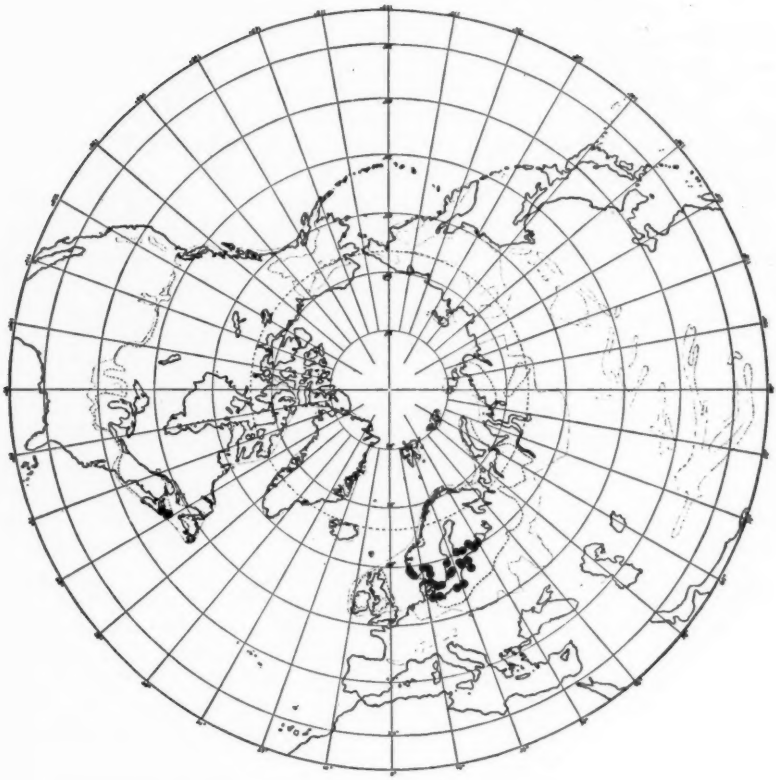


FIG. 17. Distribution area of *Polygonum oxyspermum.*

10. Polygonum exsertum Small

This tall plant of saline soils near the east coast and close to lakes on the prairies sometimes bears resemblances to *P. ramosissimum*, although it differs in many characters, particularly those of the fruit and the flowers (70, 71). Since exserted achenes much longer than the sepals are typical not only of this taxon but also of some forms of other species—and in *P. ramosissimum* may even be produced late in the season as mere modifications—Gleason (19) made the mistake of identifying this species with *P. ramosissimum* f. *atlanticum*. That this is incorrect is most easily demonstrated by the fact that *P. exsertum* is a hexaploid species with $2n = 60$ chromosomes (Fig. 12), while *P. ramosissimum* is a diploid. Hybrids between *P. exsertum* and other taxa are unknown, and all hybridization experiments have so far been unsuccessful.

11. Polygonum allocarpum Blake

This species is closely related to *P. Fowleri* and *P. prolificum*, but with a more distinctly ridged and less fleshy stem, fairly short upper internodes, greener leaves, and the fruit included or exserted even on the same branches. The species is typical of the coasts of the northeast from Newfoundland and Anticosti south to Maine and lower St. Lawrence. Also, it is known to occur on the shores of James Bay (17). It was included in *P. Fowleri* by Gleason (19), though not without hesitation. That it is a good species is shown by the fact that it is a hexaploid with $2n = 60$ chromosomes (Fig. 13).

12. Polygonum prolificum (Small) Robins.

A plant closely related to *P. ramosissimum* but differing from it not only in the smaller size but also in branching, the color of the leaves, and the form and color of the achene. It grows in saline soils although it seems to prefer more wet and marshy localities than *P. ramosissimum*. It is also a diploid plant with $2n = 20$ chromosomes (Fig. 14), but although it is the only taxon at the species level which can be crossed successfully with *P. ramosissimum*, the hybrids are highly infertile and the meiotic divisions very much disturbed owing to much decreased pairing ability of the chromosomes. Plants showing the characters of *P. ramosissimum* with, in addition, more vigorous branching, green leaves, and sepals with white margins, have been observed where both species grow together, and since these plants showed a very high degree of sterility, of the pollen grains as well as of the seeds, they may well have been natural hybrids between these species. However the cytology of these individuals has not been investigated.

13. Polygonum latum Small

A plant of roadsides and riverbanks on the prairies from Texas to the Dakotas and central Missouri, but not reaching the east coast nor Canada, this species was described by Small (in (64)). It is a tetraploid plant with $2n = 40$ chromosomes (Fig. 18), as counted on material grown from seeds of herbarium material from South Dakota.

14. Polygonum ramosissimum Michx.

This endemic bushy North American species of somewhat saline soils and roadsides is perhaps best characterized by its high growth, usually exceeding one and often reaching two meters, and by its extensive branching and yellowish rather than green appearance (17, 52). The typical race, which has been studied from several localities in Manitoba, is a diploid plant with $2n = 20$ chromosomes (Fig. 19). The sepals of the typical race become yellowish when dry, but a race described as f. *atlanticum* Robins. by Robinson (61) and found to be more common eastward has clearly red margins of the sepals also in dry condition. This race, which is hardly more than a form, following the definitions by Du Rietz (11), is also a diploid with $2n = 20$ chromosomes (Fig. 20). It hybridizes easily with the typical race and gives rise to an F_1 with only red-margined sepals and a 3 : 1 distribution in the F_2

since the difference is apparently caused by only one factor pair. It is certainly common in nature but there it cannot be distinguished from pure specimens of f. *atlanticum* without experimental studies.

15. Polygonum neglectum Bess.

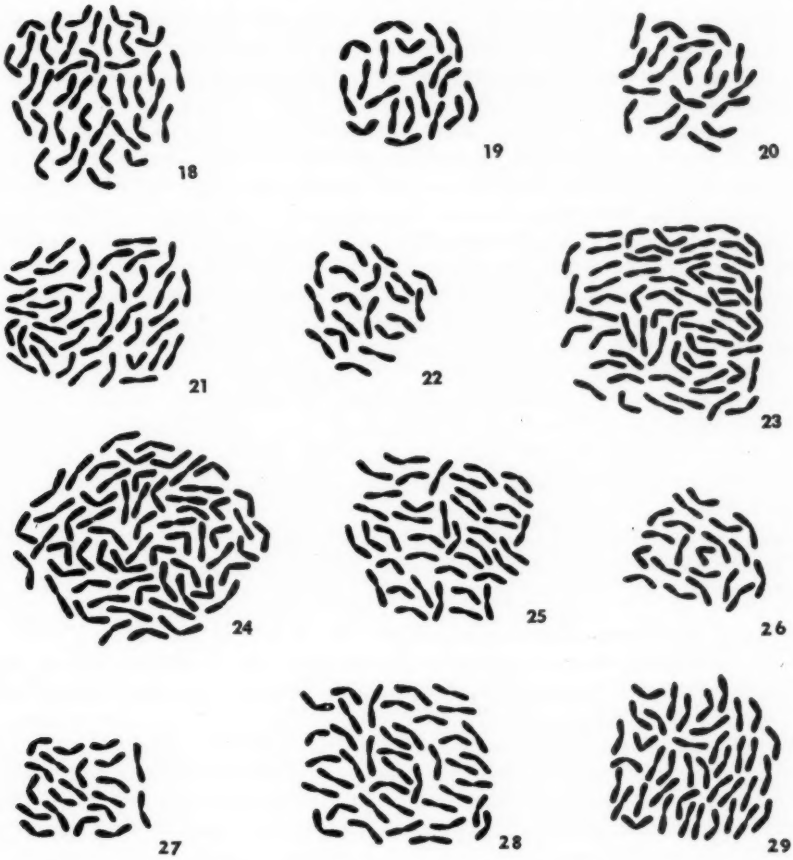
This introduced species with acute leaves, which are linear to narrowly lanceolate, resembling those of typical *P. ramosissimum*, and with red-margined sepals and dull-brown and puncticulated achenes, was described as early as 1822 by Besser (3) on material from Lithuania. Meisner (51) regarded it as a variety *angustissimum* of *P. aviculare*, Ascherson and Graebner (2) named it var. *neglectum* of that same species, while Lindman (35) included it as a variety *angustissimum* of *P. heterophyllum*. Despite its morphological distinctness it is still almost entirely neglected or misunderstood by authors of manuals (cf. 17, 19, 22). It seems to be a common weed almost all over temperate North America, and although it is especially frequent in waste places in the east, it seems to avoid the roadsides. It is a tetraploid plant with $2n = 40$ chromosomes (Fig. 21) as determined on material from southern Manitoba, and that same number was counted by Löve and Löve (42) from Sweden and reported under the name of *P. aviculare*. Hybrids between this species and the tetraploid *P. aviculare* do not seem to occur naturally, and they are rather difficult to obtain by experiment. The very pronounced sterility of the pollen of these hybrids and the absence of filled seeds strongly support the opinion that *P. neglectum* is a well-defined species clearly distinct from *P. aviculare*. The fact that the meiotic divisions of the hybrid plants usually have up to 10 bivalents and at least 20 univalents seems to indicate that of the two haplomes of the genomes (for the definition of haplome, cf. Heilbronn and Kosswig (24)) of *P. neglectum* and *P. aviculare* one is homologous but the other different. Both species are alloploid and show a very low frequency of multivalents of meiosis.

16. Polygonum rurivagum Jord.

A slender heterophyllous weed with reddish leaves and narrow, small seeds, this species was first described exactly by Jordan (in (6)) from central France. It is not mentioned in American floras, although it grows with other related weedy species in waste places and disturbed soils at least in the eastern parts of the continent. Lindman (35, 36) regarded it as a subspecies of *P. heterophyllum* despite its distinct morphological differences from that taxon, and Komarov (30) includes it without separate name in *P. aviculare*. In the recent British flora (10) it is, however, included as a species equivalent to *P. aviculare* s.str. Our Canadian material was found to be diploid with $2n = 20$ chromosomes (Fig. 22). Hybridization experiments have not yet been performed with this taxon, and natural hybrids between it and other species are not known with certainty.

17. Polygonum heterophyllum Lindm.

Described by Lindman (35) from Stockholm in Sweden, this species is a robust and very heterophyllous plant with usually lanceolate leaves, pinkish



FIGS. 18–29. Mitotic chromosomes of American *Polygonum*. Magnification × *ca.* 3500. FIG. 18. *P. latum*, $2n = 40$. FIG. 19. *P. ramosissimum* f. *ramosissimum*, $2n = 20$. FIG. 20. *P. ramosissimum* f. *atlanticum*, $2n = 20$. FIG. 21. *P. neglectum*, $2n = 40$. FIG. 22. *P. rurivagum*, $2n = 20$. FIG. 23. *P. heterophyllum* ssp. *heterophyllum*, $2n = 60$. FIG. 24. *P. heterophyllum* ssp. *boreale*, $2n = 60$. FIG. 25. *P. erectum*, $2n = 40$. FIG. 26. *P. achoreum*, $2n = 20$. FIG. 27. *P. buxiforme*, $2n = 20$. FIG. 28. *P. aviculare* ssp. *aviculare*, $2n = 40$. FIG. 29. *P. aviculare* ssp. *calcatum*, $2n = 40$.

sepals, and dull-brown and puncticulate achenes with three equally large and concave sides. It was included in *P. aviculare* as the varieties *erectum* and *vegetum* by Meisner (51) and is sometimes mentioned under these names in American manuals. The typical race, ssp. *heterophyllum*, is a common weed in North America, as elsewhere in the world. Plants from Manitoba are hexaploid with $2n = 60$ chromosomes (Fig. 23), as are specimens from Sweden (Andersson, in (44)) and Iceland (47). They often grow together with typical *P. aviculare*, and though sterile individuals with $2n = 50$ chromosomes

seem to be morphologically much closer to *P. heterophyllum* than to *P. aviculare*, they are certainly F_1 hybrids between them. Such hybrids are not frequent and have been observed only occasionally in Sweden (Andersson, in (44)) and in Manitoba.

Another race of this species occurring in North America and confined mainly to the shores in the boreal parts of the continent, is ssp. *boreale* (Lge) Löve et Löve. The leaves of this race are more fleshy, and the sepals either white- or pink-margined. Fernald (17) follows Small (70, 71) in regarding this race as the species *P. boreale* (Lge) Small and thinks that it is indigenous in the eastern parts of the country. Since this race also has $2n = 60$ chromosomes (Fig. 24) and hybridizes easily with ssp. *heterophyllum* without showing any reduction in fertility of the pollen grains or seeds or any serious disturbances of the meiotic divisions, the present writers do not agree with these views. The race ssp. *boreale* occurs also in Scandinavia, Iceland, and Greenland, so that its present area would appear to indicate an amphi-Atlantic distribution. Since the present writers (47) have, however, been able to demonstrate that this plant reached Iceland not earlier than the time of the first Norwegian settlers and certainly prior to the discovery of Vineland and Greenland, doubts as to its being indigenous in North America seem valid. It may well have been introduced to the east coast of the continent by the Icelanders and Greenlanders who visited these regions for a considerable period after the year 1000 A.D.

18. Polygonum erectum L.

A tall-growing stout plant with yellowish-green elliptic leaves differing in size on the main stem and the branches, this species was originally described by Linnaeus (37) from plants grown from seeds sent by Kalm from Philadelphia. It grows in waste places, near roadsides and on cultivated ground in eastern North America from southwest Quebec west to southern Manitoba and south to Georgia, Tennessee, and Missouri, and as a weed on the Pacific coast and probably elsewhere. It is a tetraploid species with $2n = 40$ chromosomes (Fig. 25).

19. Polygonum achoreum Blake

Separated from the last-mentioned species by Blake (4) because of differences in the color of leaves and sepals, leaf form and growth type, and fruit characters, this taxon is such a common weed in North America that Blake (4) could not decide with certainty from where it had spread originally. However, it is certainly not introduced on this continent, as suggested by Gleason (19), but most probably native in saline marshes, as suggested by Fernald (18). In addition to the morphological characters, it differs from the last species in its chromosome number, since it is a diploid with $2n = 20$ chromosomes (Fig. 26).

20. Polygonum buxiforme Small

This is a common plant from the boreal east coast to Alaska and California, in natural and disturbed habitats. It is characterized by its prostrate growth and the lanceolate-oblanceolate green leaves which are of about the same size

on all parts of the plant, but it is perhaps most certainly identified by the white-margined sepals which completely include the broad, short, and sublustrous achenes (71, 73). Although often erroneously identified, in American manuals, with *P. aviculare* var. *littorale* (Link) W. D. J. Koch, which is *P. littorale* Link from Europe, it is certainly indigenous in North America though its present area is strongly affected by the activities of man. It is clearly distinct in morphology from European *P. littorale* but identical with *P. aviculare* var. *latifolium* Michx. The chromosome number of typical *P. buxiforme* from southern Manitoba and of plants grown from seeds from the east coast was found to be $2n = 20$ (Fig. 27), while seedlings of European *P. littorale* were found to be hexaploid with $2n = 60$ chromosomes.

21. Polygonum aviculare L. s.str.

When taken in the wide sense of many authors of recent manuals, this species includes several of the species above and most of the European weeds. In its strict sense, however, it is to be retained as the correct name for the taxon named *P. aequale* Lindm. (cf. 30, 33, 35, 40) with uniformly broad leaves, and a dull-brown achene with one narrowly concave and two broadly convex sides. The typical phase of the species, or ssp. *aviculare*, is a common weed in eastern North America and fairly common all over the continent. It is a tetraploid with $2n = 40$ chromosomes (Fig. 28). This number was determined on plants from Manitoba, and it confirms the reports from Europe (29, 43, 44, 47, 57).

A much more rare race in North America is the ssp. *calcatum* (Lindm.) Thell., which is at least partially identical with *P. aviculare* var. *depressum* Meisn. It is a plant with sublustrous black and flat achenes and was originally described as a species by Lindman (34) but regarded as a good subspecies by Thellung (in (66)). It is also a tetraploid with $2n = 40$ chromosomes (Fig. 29), but it cannot be retained as a distinct species, not only because of its morphology but mainly because it hybridizes easily with ssp. *aviculare*, in natural habitats as well as experimentally, and the hybrids are either fully fertile or show a low degree of partial sterility due to some minor structural differences between some of the chromosomes; however, these differences also occur in separate populations of each subspecies.

III. Discussion

These preliminary studies on the cytogenetics of the eastern North American species of the genus *Polygonum* s.str. confirm the view that although most of these introduced or native taxa belong to the typical section of the genus, one species is sectionally distinct. This species, *P. Douglasii*, is included here only to make the chromosome reports for this part of the continent complete, but the closer relationships between this species and the other taxa of the section *Duravia* cannot be discussed at present. Also, the relationships between the two American sections of the genus would have to be studied in greater detail in the light of investigations on the cytology of the western species of section *Duravia*.

The taxa of section *Polygonum* in eastern North America are often noxious weeds whose original areas are not known with certainty. Because of their wide ecological amplitude several distinct taxa can grow together, and because of the small size of the plants and the apparently tremendous variability of the false populations produced by this mixture of taxa, taxonomists have had difficulties in separating them and even regarded this a worthless task. However, morphologists specializing in this group have distinguished between several species and varieties, and the present writers had only minor difficulties in identifying their material with these taxa described in European or American journals and manuals. Although the present treatment includes many species not recognized by American authors of recent manuals, it was not found necessary to describe a single unit as new, since the available morphological descriptions were found to be readily applicable to the various biological groups which have been distinguished by cytogenetical criteria.

Although extensive hybridization experiments have yet to be made, it is evident, from the present observations that the evolution of the species of section *Polygonum* has followed several lines. Gradual alterations of the original diploid haplome (cf. 24) have resulted in diploid species so distinct that their artificial hybrids are highly sterile because of only minor residual homology of their chromosomes. Even at the polyploid levels gradual differentiation has taken place, as shown, e.g., by the morphologically distinct races of some of the tetraploid and hexaploid species. All the polyploid species have, naturally, been produced by abrupt speciation (41), but the polyploids have received their genomes either from the same original haplome by autoploidy or from distinct haplomes by alloploidy. Although hemiautoploidy and hemialloploidy (cf. Löve and Löve (45)) seem to be common, as far as can be seen from the meiotic divisions investigated, panautoploidy has probably occurred at the original production of some of the abruptly formed species, while panalloploidy is indicated by some of the observations made. As far as can be deduced from the original distribution areas of some of the polyploids not too violently affected by the activities of man, none of them is of recent origin and some are probably very old. The subgenus as a whole is genetically stable though ecologically very adaptive.

It is remarkable how uniform the chromosomes are within the group. All the chromosomes have an almost median centromere position, and the absence of secondary constrictions makes it impossible to distinguish between any of them, at least by the methods used. Median centromere position is an indication of primitiveness within the family Polygonaceae (38).

According to Fernald (17) and Gleason (19) the most critical collective species of the group is the so-called *P. aviculare* s.lat. which includes weedy taxa mainly introduced from Europe. However, it was not difficult to demonstrate that the American representatives of this collective taxon are more correctly classified as five distinct species, two of which include two subspecies, all having been previously described in their present categories on the basis of only morphological characters. One of these species, *P. buxiforme*, seems to be confined to this continent, and another, regarded by Fernald (17)

as the indigenous species *P. boreale*, is supposed to be an early introduction and is here shown to be a subspecies of the likewise hexaploid *P. heterophyllum*. Of the taxa included in the collective species, two, *P. buxiforme* and *P. rurivagum*, are diploid, two, *P. aviculare* s.str. and *P. neglectum*, are tetraploid, while the species *P. heterophyllum* is hexaploid. Not only are the species which differ in chromosome number morphologically distinct, but the numerical chromosomal differences result in a sterility barrier; thus the classification of the diploid *P. rurivagum* and the hexaploid *P. heterophyllum* as species different from the tetraploid *P. aviculare* and *P. neglectum* is beyond discussion (cf. 40, 41, 46, 47). And since the hybrids at the tetraploid level support the classification of *P. aviculare* and *P. neglectum* as distinct species these are accepted here as such, while the taxon *P. calcatum* lacks the barrier of sterility against *P. aviculare* and is correctly classified as a subspecies of that species, if the definition of this category most clearly given by Du Rietz (11) is followed. Likewise, the taxon *P. boreale* is included as a subspecies or geographical race of *P. heterophyllum* for the same reason.

It was suggested by Fernald (17) that the difficulties in separating the taxa which he included in *P. aviculare* s.lat. might be the result of what he thought were "apparently apomictic variations". The present writers have found no indications of agamospermy of any kind within their material, but pronounced autogamy seems to be the main cause of the very low frequency of hybridization even between sympatric populations of the intraspecific races, and perhaps also between the species with the same chromosome number. The false vivipary, or the inflated and mostly empty seeds exserted from the calyx of all the species of *Polygonum* in late autumn or in other adverse conditions, has nothing to do with apomixis and is of as little taxonomical importance as are other modifications. However, the experiments could not exclude the possibility of the occurrence of a low frequency of facultative agamospermy within some races, similar to that observed in some other genera of the Polygonaceae (cf. 38, 39). But if it occurs at all, it is certainly of no taxonomical importance within this critical group.

IV. Taxonomical Review

The classification followed above is considerably different from that of recent American manuals, although it was found unnecessary to introduce any new names or combinations. Therefore, a review of the different taxa and their basic morphological differences must be given in this connection. Since at least most of the species are strongly affected by drought in the midsummer and perhaps also by differences in day length (8) so that extensive changes may take place in the appearance of the same plant from spring to autumn and make the identification almost impossible at midsummer or in the late fall, the characters used below are mainly those of the young and growing plants, or of the recovering plants in late summer. The following key is not intended to be complete in any way, and the synonymy is by no means exhaustive but given only to facilitate the identification of our taxa with those described in recent American manuals.

KEY TO THE EASTERN NORTH AMERICAN TAXA OF *Polygonum*

1. *a.* Pedicels recurved; fruits and flowers nodding ..... *P. Douglasii* Greene
   b. Pedicels straight; fruits and flowers erect or ascending 2

2. *a.* Stems erect; leaves strongly plicate 3
   b. Stems erect, ascending or prostrate; leaves flat or revolute 4

3. *a.* Achenes completely enclosed ..... *P. tenue* Michx. var. *tenue*
   b. Achenes exserted ..... *P. tenue* Michx. var. *protrusum* Fern.

4. *a.* Flowers in racemes at the leafless upper nodes of the stem or in the axils of rudimentary leaves. Pedicels as long as or longer than the flowers 5
   b. Flowers few at the nodes on usually fully leaved branches. Pedicels very short ... 7

5. *a.* Flowers closely spaced in a long, spikelike, ochreate raceme on the upper part of the stem only ..... *P. arenarium* W. & K.
   b. Flowers in elongate racemes of widely spaced and few-flowered clusters 6

6. *a.* Achenes a little exserted, longer than the calyx; upper internodes very short ..... *P. patulum* M.B. ssp. *patulum*
   b. Achenes enclosed by the calyx; upper internodes longer ..... *P. patulum* M.B. ssp. *Kitaibelianum* (Sádl.) Thell. (= *P. Bellardi* Am. auth., non All.)

7. *a.* Plant very glaucous. Sepals with broadly petaloid margins. Achenes very smooth and lustrous. Leaves minutely erose at the margins 8
   b. Plant green, bluish-green, yellowish-green, or reddish. Sepals with slightly or scarcely petaloid margins. Achenes greenish, olivaceous, brown, or blackish, lustrous to dull, often striate 9

8. *a.* Ochreae of lower nodes 4–8 mm. long, silvery-scarious, 3–5-nerved. Achenes dark. olivaceous, 4–5.5 mm. long. Sepals obovate and white- or pink-margined .. *P. Raii* Bab.
   b. Ochreae of lower nodes 6–10 mm. long, brownish with 8–16 somewhat rough nerves. Achenes black, 3–4 mm. long. Sepals oval and white-margined .... *P. glaucum* Nutt.

9. *a.* Achenes smooth and lustrous 10
   b. Achenes dull or barely sublustrous 18

10. *a.* Ochreae only slightly dissected. Prostrate or loosely spreading plants with flexible and reddish stems and leaves 11
    b. Ochreae lacerated into vein-like shreds. Erect, or decumbent-ascending plants, greenish with firm and strongly ridged stems and branches. Leaves green and distinctly veined 12

11. *a.* Ochreae 3–6 mm. long, almost veinless. Leaves succulent, elliptic and narrowly elliptic-obovate, obtuse, and slightly veined. Achenes olive-brown, 3–4 mm. long ..... *P. Fowleri* Robins. (= *P. buxifolium* Nutt., non M.B.)
    b. Ochreae 10–15 mm. long, with prominent veins. Leaves linear-oblanceolate, acute, submembranaceous, with evident veins. Achenes greenish, 6–7 mm. long ..... *P. oxyspermum* Mey. & Bge. (= *P. acadiense* Fern.)

12. *a.* Ochreae of upper leaves 5–7 mm. long, extending much beyond the flowers ..... *P. autumnale* Brenckle
    b. Ochreae of upper leaves 1–3 mm. long, shorter than the flowers 13

13. *a.* Achenes lanceolate, pale green, strongly exserted from the calyx ... *P. exsertum* Small
    b. Achenes olivaceous to dark brown, either included or exserted 14

14. *a.* Pedicels included within the sheathing ochreae 15
    b. Pedicels when mature exserted above the sheathing ochreae. Stem and branches erect, their upper leaves reduced 16

15. *a.* Stem decumbent with prostrate branches. Leaves oval, oblong, narrowly elliptic or elliptic-obovate. Achenes inequilateral, olivaceous ..... *P. allocarpum* Blake
    b. Stem and branches ascending. Leaves linear-oblong. Achenes ovoid, dark brown ..... *P. prolificum* (Small) Robins.

16. *a.* Plant branched at the base, up to 100 cm. tall, the branches spreading. Lower leaves spathulate to oblong, fairly deciduous ..... *P. latum* Small
b. Plant branched above, up to 200 cm. tall, the branches ascending. Leaves narrowly linear to lanceolate or oblanceolate, acute, those on the lower part of the stem persistent 17

17. *a.* Sepals yellowish-green with yellow margins, drying yellowish ..... *P. ramosissimum* Michx. f. *ramosissimum*
b. Sepals roseate-margined, also when dry ..... *P. ramosissimum* Michx. f. *atlanticum* Robins.

18. *a.* Leaves acute, linear-lanceolate to linear 19
b. Leaves obtuse, oval, elliptic, obovate to lanceolate 20

19. *a.* Achenes about 3 mm. long, lanceolate. Stem slender and weak, prostrate and homophyllous, up to 100 cm. long. Ochreae up to 15 mm. long, white and transparent. Medium-sized flowers in clusters ..... *P. neglectum* Besser (= *P. aviculare* var. *angustissimum* Meisn.)
b. Achenes usually 1–1.5 (rarely up to 2) mm. long, almost ovoid. Stems slender, decumbent to ascending, heterophyllous, up to 20–30 cm. long. Ochreae up to 10 mm. long, hyaline and transparent at the tip, brownish-red below. Flowers few or single at the nodes, small ..... *P. rurivagum* Jord. (= *P. aviculare* var. *rurivagum* Gentil.)

20. *a.* Distinctly heterophyllous, so that the leaves of the flowering branches are considerably smaller than those of the stem. Sepals broad, green, with white to pink petaloid margins. Achenes ovoid with three equally large and concave sides, reddish-brown to blackish 21
b. Homophyllous, or slightly heterophyllous, so that the leaves of the flowering branches are only slightly smaller than those of the stem 22

21. *a.* Stems robust, with spreading branches, not matted. Leaves ovate-lanceolate to lanceolate, green and not fleshy ..... *P. heterophyllum* Lindm. ssp. *heterophyllum* (= *P. aviculare* vars. *vegetum* and *erectum* Meisn.)
b. Stems loosely ascending to prostrate, little branched, matted. Leaves long-petioled, oblong-obovate to spathulate, slightly fleshy, reddish or dark green ..... *P. heterophyllum* Lindm. ssp. *boreale* (Lge) Löve & Löve (= *P. boreale* (Lge) Small)

22. *a.* Leaves and sepals yellowish-green. Leaves elliptic. At maturity the pedicels are exserted from the fibrous ochreae ..... *P. erectum* L.
b. Leaves bluish-green. Margins of sepals pink or white or even red to purplish. Pedicels mostly included. Achenes inequilaterally trigonous, with two broadly convex and one narrowly concave sides 23

23. *a.* Plant ascending, decumbent-ascending, or erect, with crowded broad, elliptic or obovate leaves which are broadly rounded above. Achenes olivaceous ..... *P. achoreum* Blake
b. Plant prostrate, loosely spreading or erect, with mainly scattered elliptic to oblong or oblanceolate to lanceolate leaves. Achenes dark brown to blackish 24

24. *a.* Leaves thick and firm or subcoriaceous, lanceolate to oblanceolate, rarely oblong, always obtuse. Achenes minutely puncticulate ..... *P. buxiforme* Small (= *P. aviculare* var. *littorale* auct. Am., non (Link) Koch)
b. Leaves relatively thin, oblanceolate or elliptic, obtuse 25

25. *a.* Achenes minutely granular-striate, dull, their concave side narrow but distinct ..... *P. aviculare* L. ssp. *aviculare* (= *P. aequale* Lindm., *P. monspeliense* Thieb.)
b. Achenes smooth, sublustrous, their concave side so narrow that the fruit appears quite flat or two-sided ..... *P. aviculare* L. ssp. *calcatum* (Lindm.) Thell. (= *P. aviculare* var. *depressum* Meisn.)

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References

1. ADANSON, M. Familles des plantes. Paris. 1763.
2. ASCHERSON, P. and GRAEBNER, P. Synopsis der mitteleuropäischen Flora. Bd. IV. Leipzig. 1908–1913.
3. BESSER, W. Enumeratio plantarum huisque in Volhynia Podolia, etc. collectarum. Wilno. 1822.
4. BLAKE, S. F. Two new Polygonums from New England. Rhodora, 19 : 232-235. 1917.
5. BONGARD, M. Observations sur la végétation de l'île de Sitcha. Mém. Acad. Sci. St. Pétersbourg, 6 : 119-178. 1833.
6. BOREAU, A. Flore du centre de la France. Tome II, Ed. 3. Paris. 1857.
7. BÖRNER, C. Botanisch-systematische Notizen. Abhandl. Naturw. Ver. Bremen, 21 : 245-282. 1913.
8. BRENCKLE, J. F. Notes on *Polygonum* (*Avicularia*). Bull. Torrey Botan. Club, 68 : 491-495. 1941.
9. BRIQUET, J. International rules of botanical nomenclature. Jena. 1935.
10. CLAPHAM, A. R., TUTIN, T. G., and WARBURG, E. F. Flora of the British Isles. Cambridge University Press, London. 1952.
11. DU RIETZ, G. E. The fundamental units of biological taxonomy. Svensk Botan. Tidskr. 24 : 333-428. 1930.
12. FERNALD, M. L. Some North American relatives of *Polygonum maritimum*. Rhodora, 15 : 68-73. 1913.
13. FERNALD, M. L. A new maritime *Polygonum* from Nova Scotia. Rhodora, 16 : 187-189. 1914.
14. FERNALD, M. L. The Gray Herbarium expedition to Nova Scotia 1920. Part II. Noteworthy vascular plants collected in Nova Scotia, 1920. Rhodora, 23 : 184-195, 223-245, 257-278, 284-300. 1920.
15. FERNALD, M. L. Persistence of plants in unglaciated areas of boreal America. Mem. Am. Acad. Arts Sci. 15 : 241-342. 1925.
16. FERNALD, M. L. The seventh century of additions to the flora of Virginia. Part II. Range extensions, technical notes and revisions. Rhodora, 44 : 376-405, 416-452. 1943.
17. FERNALD, M. L. Gray's manual of botany. 8th ed. American Book Company, New York. 1950.
18. FERNALD, M. L. Three additions to the flora of Nova Scotia. Rhodora, 52 : 18-19. 1950.
19. GLEASON, H. A. Illustrated flora of the northeastern United States and adjacent Canada. New York Botanical Garden, N.Y. 1952.
20. GREENE, E. L. Certain Polygonaceous genera. Leaflet Botan. Observation and Criticism, 1 : 17-50. 1904.
21. GROSS, H. Beiträge zur Kenntnis der Polygonaceen. Botan. Jahrb. 49 : 234-339. 1913.
22. GROSS, H. Remarques sur les Polygonées de l'Asie Orientale. Bull. géograph. botan. 4 (22) : 7-32. 1913.
23. HEDBERG, O. Pollen morphology in the genus *Polygonum* L. s.lat. and its taxonomical significance. Svensk Botan. Tidskr. 40 : 371-404. 1946.
24. HEILBRONN, A. and KOSSWIG, C. Principia Genetica. J. Unified Sci. (Erkenntnis), 8 : 229-255. 1939.
25. HULTÉN, E. Flora of Alaska and Yukon. I–X. Acta Univ. Lundensis, N. F. II, 37-46 : 1–1902. 1941–1950.
26. HULTÉN, E. Atlas över växternas utbredning i Norden. Stockholm. 1950.
27. HYLANDER, N. Förteckning över Nordens växter. I. Kärlväxter. Lund. 1955.
28. JARETZKY, R. Beiträge zur Systematik der Polygonaceen unter Berücksichtigung des Oxymethylanthrachinon-Vorkommens. Feddes Repertorium, 22 : 49-83. 1925.
29. JARETZKY, R. Histologische und karyologische Studien an Polygonaceen. Jahrb. wiss. Botan. 69 : 357-490. 1928.
30. KOMAROV, V. L. *Polygonum* in Flora SSSR, 5 : 594-661, 672-701. 1936.
31. LEDEBOUR, C. F. Index seminum horti dorpatensis. Dorpati. 1822.
32. LEDEBOUR, C. F. Flora rossica. Vol. 3. Stuttgartiae. 1851.
33. LEMBKE, J. Étude sur les menues espèces appartenant à l'espèce collective *Polygonum aviculare* L. Acta Soc. Botan. Polon. 19 : 201-207. 1948.
34. LINDMAN, C. A. M. *Polygonum calcatum* nov. spec. inter *Avicularia*. Botan. Notiser, 139-144. 1904.
35. LINDMAN, C. A. M. Wie ist die Kollektivart *Polygonum aviculare* zu spalten ? Svensk Botan. Tidskr. 6 : 673-696. 1912.
36. LINDMAN, C. A. M. Svensk fanerogamflora. Andra upplagan. Stockholm. 1926.
37. LINNAEUS, C. Species plantarum. Holmiae. 1753.
38. LÖVE, Á. Cytogenetic studies on *Rumex* subgenus *Acetosella*. Hereditas, 30 : 1-136. 1943.
39. LÖVE, Á. Agamospermy in *Acetosa*. Hereditas, 35 : 390-393. 1949.
40. LÖVE, Á. Taxonomical evaluation of polyploids. Caryologia, 3 : 263-284. 1951.

41. LÖVE, Á. The foundations of cytotaxonomy. VIII[e] Congrès Intern. Botan. Paris, Rapp. et Comm. Sect. 9-10 : 59-66. 1954.
42. LÖVE, Á. and LÖVE, D. Cytotaxonomic studies on boreal plants. I. Some observations on Swedish and Icelandic plants. Kgl. Fysiograf. Sällskap. Lund Förh. 12 (6) : 1-19. 1942.
43. LÖVE, Á and LÖVE, D. Chromosome numbers of Scandinavian plant species. Botan. Notiser, 19-59. 1942.
44. LÖVE, Á and LÖVE, D. Chromosome numbers of Northern plant species. Iceland University Inst. Appl. Sci., Dept. of Agric., Rept. B, 3 : 1-131. 1948.
45. LÖVE, Á. and LÖVE, D. The geobotanical significance of polyploidy. I. Polyploidy and latitude. Portug. acta biol. (A), R. B. Goldschmidt Vol. : 273-352. 1949.
46. LÖVE, Á and LÖVE, D. Cytotaxonomical studies on the northern bedstraw. Am. Midland Naturalist, 52 : 88-105. 1954.
47. LÖVE, Á and LÖVE, D. Cytotaxonomical conspectus of the Icelandic flora. Acta Horti Gotoburgensis, 20 : 1-290. 1956.
48. LÖVE, Á and SARKAR, N. Cytotaxonomy and sex determination of *Rumex paucifolius*. Can. J. Botany, 34 : 261-268. 1956.
49. MARIE-VICTORIN, FRÈRE. Flore laurentienne. Montréal. 1935.
50. MEISNER, C. F. Monographiae generis Polygoni prodromus. Genevae. 1826.
51. MEISNER, C. F. Polygonaceae. *In* De Candolle: Prodromus systematis naturalis, etc. 14 : 28-186. 1856.
52. MICHAUX, F. A. Flora Boreali-Americana. Paris. 1803.
53. MILLER, P. The Gardeners' Dictionary. Abridged. 4th ed. London. 1754.
54. MILLER, P. The Gardeners' Dictionary. 8th ed. London. 1768.
55. NAKAI, T. Tadervi no shinburniko. ("A new classification of Linnaean *Polygonum*"). Rizakkai, 24. 1926. (In Japanese, cf. Hedberg (23) and Steward (78)).
56. NUTTALL, T. The genera of North American plants and a catalogue of the species to the year 1817. Philadelphia. 1818.
57. PÓLYA, L. Chromosome numbers of certain alkali plants. Arch. Biol. Hung. Ser. II, 18 : 145-148. 1948.
58. RECHINGER, K. H. The North American species of *Rumex*. Field Museum Nat. History, Botan. Ser. 17 : 1-151. 1937.
59. RECHINGER, K. H. Rumices asiatici. Vorarbeiten zu einer Monographie der Gattung *Rumex*. VII. Candollea, 12 : 9-152. 1949.
60. RECHINGER, K. H. Monograph of the genus *Rumex* in Africa. Botan. Notiser, Suppl. 3 (3) : 1-114. 1954.
61. ROBINSON, B. L. The New England Polygonums of the section *Avicularia*. Rhodora, 4 : 65-73. 1902.
61*a*. RODRIGUES, J. E. de M. Contribuição para o conhecimento cariológico das halófitas e psamófitas litorais. Disertação, Coimbra, 1-210. 1953.
62. ROLAND, A. E. The flora of Nova Scotia. Proc. Nova Scotian Inst. Sci. 21 (3) : 1-552. 1944–1945.
63. RYDBERG, P. A. Flora of the Rocky Mountains and adjacent plains, etc. 2nd ed. New York Botanical Garden, N.Y. 1922.
64. RYDBERG, P. A. Flora of the prairies and plains of central North America. New York Botanical Garden, N.Y. 1932.
65. SAMUELSSON, G. *Polygonum oxyspermum* Mey. et Bge. und *P. Raii* Bab. ssp. *norvegicum* Sam. n. ssp. Acta Horti Bergiani, 11 : 68-80. 1931.
66. SCHINZ, H. and KELLER, R. Flora der Schweiz. II. Teil: Kritische Flora. Dritte Auflage. Zürich. 1914.
67. SCOGGAN, H. J. The flora of Bic and the Gaspé Peninsula, Québec. Natl. Museum Can. Bull. 115 : 1-399. 1950.
68. SMALL, J. K. A preliminary list of American species of *Polygonum*. Bull. Torrey Botan. Club, 19: 351-370. 1892.
69. SMALL, J. K. Further notes on American species of *Polygonum*. Bull. Torrey Botan. Club, 20 : 213-217. 1893.
70. SMALL, J. K. New and interesting species of *Polygonum*. Bull. Torrey Botan. Club, 21 : 168-173. 1894.
71. SMALL, J. K. Monograph of the northern American species of the genus *Polygonum*. Mem. Dept. Botany, Columbia College, 1 : 1-183. 1895.
72. SMALL, J. K. Flora of the southeastern United States, etc. New York. 1903.
73. SMALL, J. K. Studies in North American Polygonaceae. II. Bull. Torrey Botan. Club, 23 : 51-57. 1906.
74. SMALL, J. K. Flora of the southeastern United States, etc. 2nd ed. New York. 1913.
75. SMALL, J. K. Manual of the southeastern flora, etc. New York. 1933.
76. SOÓ, R. A Magyar Növényvilág Kézikönyve. I–II. Budapest. 1951.
77. STEVENS, O. A. Handbook of North Dakota plants. Fargo. 1950.
78. STEWARD, A. N. The Polygonaceae of eastern Asia. Contribs. Gray Herbarium, 88 : 1-129. 1930.

THE VEGETATION OF NORTHERN MANITOBA

I. STUDIES IN THE SOUTHERN SPRUCE FOREST ZONE[1]

By J. C. Ritchie

Abstract

It is pointed out that there are available no ecological accounts of the vegetation of Northern Manitoba and the surrounding regions. After brief accounts of the topography, geology, and climate, the communities which were met with in two areas of study are described. It is shown that the predominant and stable forest of mesic sites is dominated by *Picea mariana*, with a ground vegetation composed chiefly of weft-form mosses. In areas which have been burned various subseral variants of this forest are present. *Pinus banksiana*, as well as being a seral dominant of mesic sites, dominates various forests of outcrop ridges, sand plains, and eskers. *Picea glauca* is rare in the area, occurring only on those sites which show exceptionally favourable conditions of habitat. The vegetation of this region conforms with descriptions of the Southern Spruce Forest Zone of Eastern Canada, which classification might well be extended westwards at least as far as northwest Manitoba. There is some evidence that the dry climate which prevails west of the region, and for which there is slight evidence here, has influenced the flora and vegetation of the region. A description is given of a highly local community of *Betula papyrifera* var. *neoalaskana* on organic ridges which appears never to have been recorded previously for North America.

I. Introduction

The grouping into forest types of the vegetation of Canada by Halliday (11) has provided the basis for several subsequent ecological studies. The description and delimitation of vegetation zones in Eastern Canada has been considerably advanced by the investigations of Hare (13, 14), Hare and Taylor (15), Hustich (18, 19, 21, 22), Marr (30), and Rousseau (47), and in the northwest there are similar studies by Lewis, Dowding, and Moss (28), Moss (32, 33, 34, 35), Raup (38, 40, 41, 42, 43, 44), and Raup and Denny (45). However there has been no comparable examination of the plant communities which occur in the considerable area of forested boreal Canada which lies between the southwest shores of Hudson Bay and the northwest regions of Saskatchewan. As a contribution to the establishment of more detailed ecological knowledge of this region, the present series of investigations is designed to explore the vegetation regions of Manitoba which lie to the immediate north of the southern limit of Halliday's (11) Northern Coniferous Zone, shown in Fig. 1. And the present account is concerned with a small area which lies at the northern limit of this zone. Field studies were carried out in the regions of MacBride Lake (56° 50′ N., 99° 53′ W.) and Tod Lake (56° 35′ N., 101° 47′ W.) (Fig. 1), and their object was to provide detailed descriptions of the plant communities of the relatively small areas which are mapped in Figs. 2 and 3. From the results of such a field study it should be possible, using the established techniques of the interpretation of aerial photographs, to extend the survey into adjacent areas which were not visited,

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Contribution from the Department of Botany, University of Manitoba, Winnipeg, Canada.

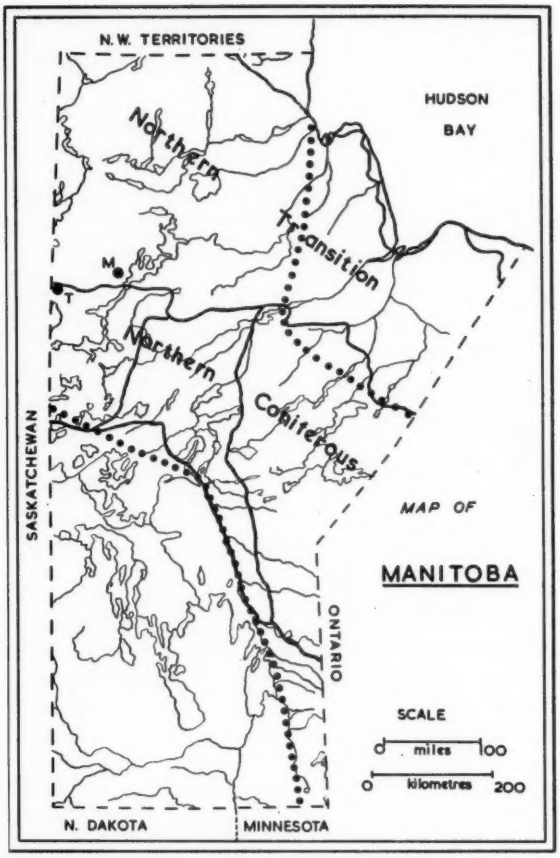


FIG. 1. The locations of Tod Lake (T) and MacBride Lake (M) are shown. The area between the dotted lines marks the position and extent of the Canadian Shield in Manitoba. The heavy continuous lines indicate the limits of Halliday's major divisions of the coniferous forest.

thereby obtaining a representative description of the vegetation zones of the general region. The present account is concerned only with the detailed ecological descriptions and the latter topic will be made available in a subsequent report.

II. Physiography, Geology, and Climate

Throughout the entire region relief is low, seldom exceeding 50 m. above the level of the lakes. Both the MacBride Lake and Tod Lake areas are characterized by low, undulating ridges of glacial drift, with numerous out-

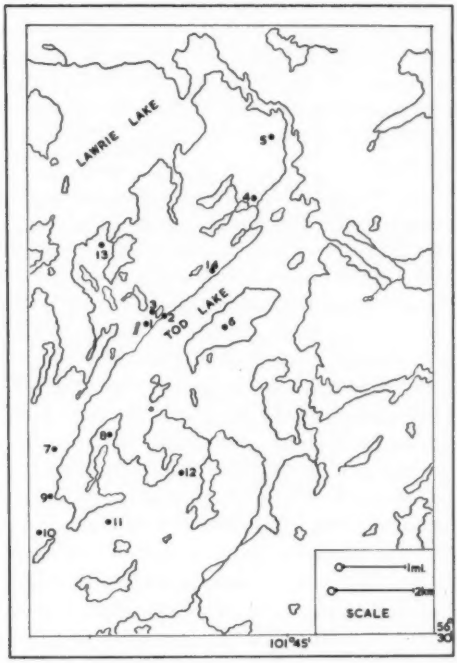


FIG. 2. A map of the immediate precincts of Tod Lake showing the positions of the stations (referred to in the text) at which detailed studies were made.

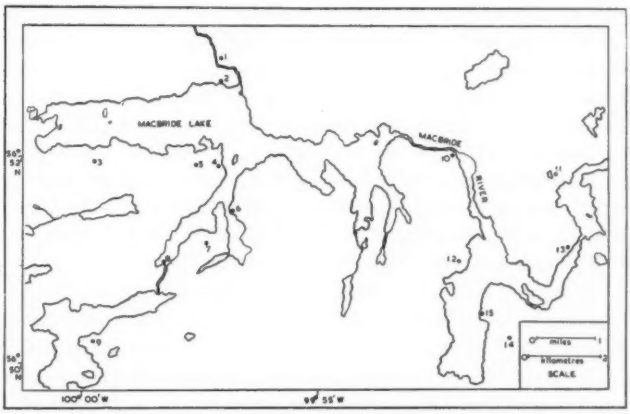


FIG. 3. A map of the region of MacBride Lake and MacBride River showing the positions of the stations at which particular studies were carried out and to which reference is made in the text.

crops of bedrock. The valleys and depressions are filled by lakes, muskegs, and bogs. The drainage systems of both areas flow eastwards, effected by the MacBride River and the Lawrie River respectively. Accounts of the geology of the two regions are available, the MacBride Lake area by Kilburn (24) and the Tod Lake area is included in the district described by Milligan (31). All the rocks of both areas are of pre-Cambrian age. Glaciation was from the north-northeast.

In the region of MacBride Lake, lying to the northwest of the lake, there is an extensive area of glacial clay which forms undulating plains which are unbroken by any outcrops; it bears an interesting vegetation type which will be considered below. South of MacBride Lake there is a large esker which runs in a north/south direction (map in Kilburn (24)), and a smaller one was noted on the north shore. The area in the immediate vicinity of Tod Lake includes an extensive sand plain at the south end of the lake. The typical low relief of this region is broken along the north shore of Tod Lake where a ridge with low cliffs, 60–75 m. above the level of the lake, provides a highly unusual feature of topography. This is caused by ". . . a complex zone of faulting . . ." at the contact between two series of sediments (Milligan (31, pp. 3–4)). The relatively abrupt relief of this ridge has produced a series of habitats which bear plant communities of considerable interest, referred to in subsequent sections.

As Hare (13) has pointed out, there is little use to which ecology can put the raw data of climate. However Thornthwaite (54) has introduced a system by which a more direct approach to the role of climatic factors in ecological problems is possible. These methods and their applications have been discussed and implemented by Sanderson (49, 50) and Hare (13, 14). Using the first element in Thornthwaite's classification, Sanderson (49) has mapped the zones of thermal efficiency in Canada, based on the average evapotranspiration; this is defined as "the amount of water that would be transferred from the soil to the atmosphere by evaporation and transpiration if it were constantly available in optimum quantity" (Sanderson (49, p. 501)). The region of the present investigation lies within the C_2' or warm microthermal zone. Thornthwaite (54) further defined certain moisture regions, on the basis of water deficiency, water surplus, and water need (= evapotranspiration). These regions have been mapped by Sanderson (49) and the region of the present study lies at the western edge of the C_2 or moist subhumid region; that is, it is just within the western boundary of the moist climates which prevail in eastern boreal Canada.

Considering the Shield area of Manitoba (Fig. 1), the northwest region, centered approximately round the areas of study, shows the greatest values for indices of drought; it has the lowest values for water surplus and the highest for water deficiency (Sanderson (50)). The possible ecological significance of this tendency towards relative dryness of climate will be considered later in this paper. Certain climatic data for the region are summarized in Table I. These have been extracted from Sanderson (50) and Thomas (53).

TABLE I

CLIMATIC DATA FOR THE AREAS OF STUDY

Potential evapotranspiration	± 42 cm.
Water surplus	± 5 cm.
Moisture deficiency	± 7 cm.
Mean annual temperature	− 4° C.
Mean July daily temperature	16° C.
Mean January daily temperature	−28° C.
Mean annual total precipitation	40 cm.
Mean annual total rainfall	30 cm.

III. The Methods of Study

Some attempt was made in the field to select particular stands of vegetation for study by a preliminary scrutiny of aerial photographs; in this way as representative a survey as possible was made. For each stand, data of topography, a description of the soil profile (in more or less detail), a complete annotated list of species of vascular plants, bryophytes, and lichens, and miscellaneous data about status, zonation, and other topics, were recorded. For each vegetation type one or more stands was studied in greater detail, using simple qualitative and quantitative methods of description and analysis. This involved the setting out of 10 × 5 m. quadrats, randomly selected, in which all trees and shrubs were counted, their heights estimated and their trunk diameters at 1 m. from ground level measured; the lower synusiae of vegetation were then examined, the relative abundance of the component species being indicated by the standard frequency symbols. In those stands where more detailed studies were made, line transects were used to provide further descriptions of the community structure; this was accomplished by plotting the position of each individual on the line, estimating its height and width of canopy, and making an outline sketch of the spatial arrangement of all the trees and shrubs along the transect. From the floristic descriptions of all stands of each vegetation type Presence Class values (after Braun-Blanquet (3)) have been determined.

Subsequent laboratory studies of vertical aerial photographs of the areas of this enquiry have enabled detailed keys to be constructed by which various vegetation types can be identified on the photographs; in this way, by stereoscopic examination of normal size and ×-4 enlargements, various vegetation maps of the areas have been prepared. The results and methods of this work will be reported in detail in a subsequent paper.

During the course of the field work, specimens of vascular plants, bryophytes and lichens were collected. At present they are included in the author's private collection; a duplicate set of the bryophytes is in the National Museum of Canada, Ottawa, and a duplicate set of the lichens has been deposited in the Herbarium of the Department of Botany, University of Wisconsin.

IV. A Descriptive Account of the Vegetation

Before proceeding with the formal description of the several types of vegetation which were encountered in this region it is necessary to make clear the sense in which certain terms are used. *Muskeg* is used in the precise sense of Hustich (19, p. 12 and 22, p. 15); that is, it describes a bog forest which has a single tree stratum of *Picea mariana*, and a hummocked ground vegetation of mosses—chiefly species of *Sphagnum*—which bears a varied shrub and herb community dominated by *Ledum groenlandicum*. Most North American authors use muskeg to include all communities on wet, acid, organic substrata, such as Dansereau (in Kimble and Good (25)), Lewis, Dowding, and Moss (28), Moss (34), and Raup (42). From the botanical point of view it ceases to be meaningful when given the even broader meaning of Küchler (26) by which it includes swamp vegetation; Dansereau and Segadas-Vianna (8) have made clear the basic distinctions between swamp and bog. In the use of *peat bog* Hustich (19) will be followed, and the primary distinction between this type and muskeg is that there is no tree stratum in the former.

It should be stressed that the terms which are used here to designate certain forest types—for example, the 'closed spruce/willow/alder mixed forest'—are of a temporary nature. Until a more thorough examination of a large number of stands over a greater area is available, the use of any particular system of classification would be scarcely justified. The terms which have been used, based on the simplest possible criteria, should be regarded merely as convenient abbreviations of the descriptions. Homologies with more exact terms of other authors will be indicated. There is a considerable need for thorough descriptive accounts of the ecology of the vegetation of this central region of boreal and subarctic Canada; if such data were available the application of various rigid systems of classification might be meaningful (cf. Cain (4 and 5)).

The Spruce Forest and Related Types

Closed Black Spruce Forest

In all parts of the region where there is a well developed, moderately drained mineral soil, and where there is no evidence of recent fire, a forest is present which is dominated solely by *Picea mariana*. Several sites bearing such a forest were examined in detail, and the following description of the stand on the south shore of MacBride Lake, at Station 4 (Fig. 3) is completely representative.

The trees are 14–20 m. tall, apparently mature, with trunks of 20–30 cm. in diameter at 1 m. from ground level. There is no stratification in the community, and only under local breaks in the canopy caused by windfalls are other trees or tall shrubs present. The soil profile reveals an upper horizon of fibrous partially decomposed moss humus of 6 cm. depth, immediately below the carpet of living mosses, which is 3–5 cm. in depth. Below the H_1 horizon is a shallow layer of slightly compacted, well decomposed humus, overlying a reddish-brown, smooth textured clay-loam horizon of 5–8 cm.

depth. At 25–30 cm. there is a broad horizon of light brown clay-loam extending for 40 cm. There is some evidence of gleying at 50–60 cm. in areas of slightly impeded drainage. Permafrost was encountered at this time (July) at a depth of 1.5 m.

Scattered throughout the forest are erect, tall stumps of *Betula papyrifera* var. *neoalaskana*, 2–3 m. tall, with the wood completely decayed but held upright by the tough, resistant outer cylinder of bark (Fig. 10). These relics provide a useful clue to the stages of development of the spruce forest and they will be referred to below in a discussion of seral relationships. There is some evidence of spruce regeneration in the forest, and a few of the 10 × 5 m. quadrats which were examined showed several young trees of 40–100 cm. height.

The ground vegetation is uniform and relatively poor in species. *Ledum groenlandicum* and *Vaccinium vitis-idaea* ssp. *minor* occur in local abundance, and *Lycopodium annotinum*, *Geocaulon lividum*, and *Rosa acicularis** are widespread but never locally abundant. On well drained slopes—particularly

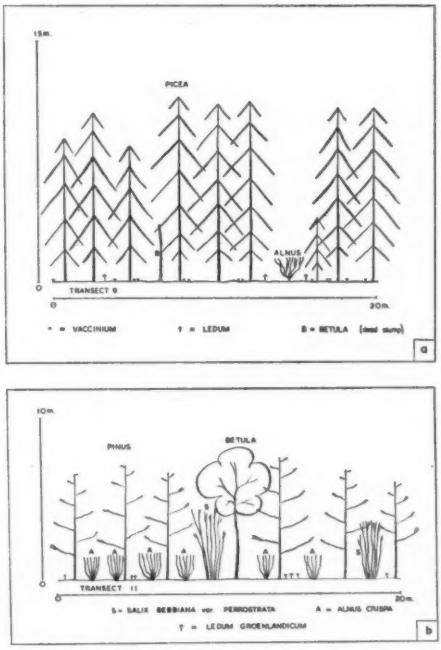


FIG. 4*a*. A profile diagram of the mature closed black spruce forest which is summarized in Table II. The species shown are *Vaccinium vitis-idaea* ssp. *minor*, *Ledum groenlandicum*, *Betula papyrifera* var. *neoalaskana*, and *Alnus crispa*.

FIG. 4*b*. A profile diagram of the open seral pine forest described in Table V. The tree species are *Pinus banksiana* and *Betula papyrifera* var. *neoalaskana*.

**Used throughout in sensu lato, probably R. sayii Schwein.*

TABLE II

THE CLOSED BLACK SPRUCE FOREST

Species	Quadrats (10 × 5 m.) 20	21	22	23	Presence class (8 sites)
Picea mariana	12(12–25 m.)* 4(4 m.) 1(1 m.)	19(14–20 m.) 2(3 m.) 5(1 m.)	10(12–18 m.) 3(2 m.) 3(½ m.)	11(10–15 m.) 5(2 m.) 1(½ m.)	5
Betula papyrifera var. *neoalaskana*	2 (dead)	–	1 (dead)	–	3
Alnus crispa	–	–	l	–	4
Ledum groenlandicum	o	f	f	f	5
Lycopodium annotinum	o	–	–	–	4
Geocaulon lividum	lf	o	–	–	5
Rosa acicularis	–	–	r	–	4
Vaccinium vitis-idaea ssp. *minor*	f	f	f	f	5
Linnaea borealis ssp. *americana*	–	–	–	r	3
Petasites palmatus		–	–	r	2
Ceratodon purpureus	lf	o	o	–	4
Dicranum bergeri	f	f	f	o	5
D. fuscescens	o	o	–	r	3
D. rugosum	f	f	o	f	5
Pohlia nutans	lf	o	o	o	5
Pleurozium schreberi	d	d	d	d	5
Hylocomium splendens	a	f	f	a	5
Ptilium crista-castrensis	la	la	ld	o–f	5
Ptilidium ciliare	lf	o	o	o	5
Peltigera aphthosa	o	o	o	f	5
Cladonia alpestris	–	–	–	o, la	5
C. rangiferina	o	–	o	o	4

**12(12–25 m.) = 12 individuals, between 12 m. and 25 m. tall.*

those with a southern aspect—the following species are characteristic: *Equisetum scirpoides*, *Chamaenerion angustifolium*,* *Chamaepericlymenum canadense*,† *Ramischia secunda*,‡ *Mertensia paniculata*, *Linnaea borealis* ssp. *americana*, and *Petasites palmatus*. By far the greater area of the ground is covered by a dense carpet of mosses, most commonly belonging to the weft growth-form type (Gimingham and Robertson (10)). The dominant moss is *Pleurozium schreberi;* it is associated with *Hylocomium splendens*, *Dicranum rugosum*, *D. fuscescens*, *D. bergeri*, and the thalloid lichen *Peltigera aphthosa*. In local abundance in shaded, damp sites is *Ptilium crista-castrensis;* and *Pohlia nutans*, *Ceratodon purpureus*, *Ptilidium ciliare*, and *Cladonia glauca* occur exclusively at the bases of spruce trees, extending up the trunk for short distances. The mineral soil which is exposed by fallen spruces is invariably colonized by a dense carpet of *Polytrichum piliferum*. *Cladonia alpestris* and *C. rangiferina* assume local dominance in areas of increased illumination, usually associated with *Alnus crispa* which occupies gaps left by fallen spruces. The general aspect of this forest type is illustrated by Fig. 8, and the physiognomy by the line transect depicted in Fig. 4*a*. Table II summarizes the quadrat studies which were conducted at the site which has been described above.

In areas where drainage becomes increasingly impeded there is a transition zone from this spruce forest to muskeg, and thence, in many cases, to peat

* = *Epilobium angustifolium.*
† = *Cornus canadensis.*
‡ = *Pyrola secunda.*

bog. These zonal changes will be described in detail below, in the section which is concerned with muskeg and peat bog.

One of the most obvious vegetational features of this region is that many areas of identical topography and substratum are covered with forest types which differ in both structure and floristic composition. Many of these diverse forest types are found in mesic habitats which are similar to that of the spruce forest described above, and in fact many abut directly on areas of continuous black spruce forest, the narrow zone of transition being marked by no apparent changes in habitat factors. Both field observations and detailed study of vertical aerial photographs indicate that these differences are due to the differential effects of forest fires; the various types of forest will be described below and their possible seral relationships will be discussed.

The Closed Spruce/Willow/Alder Mixed Forest

Within this group are included the closed forests on moderately drained, well developed soils, whose phanerophytic strata are dominated by *Picea mariana*, *Salix bebbiana* var. *perrostrata*, and *Alnus crispa* respectively. This forest type is of frequent occurrence in the general area of study and it forms continuous stands on ridges, slopes, and clay plains: it is readily identified on aerial photographs by the isolated crowns of white birch in a stippled matrix of conifers. Several stands were studied in detail and the following description of the large stand on the west shore of MacBride River (Station 12, Fig. 3) is typical. The uppermost stratum is formed of three species, the dominant *Picea mariana*, with *Pinus banksiana* and *Betula papyrifera* var. *neoalaskana*. The pine and birch occur only occasionally but they are constant features of the vegetation. This stratum occupies the 4–10 m. level. The second tree layer consists solely of *Salix bebbiana* var. *perrostrata*, which is common throughout, occurring as tall, dense, shrubby trees at 3–6 m. There is a distinct tall shrub layer at 1–2 m., formed by the abundant *Alnus crispa* together with young spruce trees. A low shrub stratum can be distinguished at 15–60 cm., consisting of *Ledum groenlandicum;* it is associated with other smaller shrubs, in particular *Rosa acicularis*, *Ribes glandulosum*, and *Vaccinium vitis-idaea* spp. *minor*. The spatial relationships of the three phanerophytic strata are depicted in Fig. 5.

A cursory examination of the soil profile of this locale indicated that it is of the heavy clay-loam type. There is an upper horizon, 1–4 cm. in depth, of compacted humus, a humous-stained loamy horizon of 8–12 cm., and an underlying horizon of clay-loam of 30–50 cm. The surface is uneven, consisting of hummocks and intervening hollows; in the latter there is evidence of impeded drainage—a deeper humous layer and a preponderance of mesophytic mosses.

The ground vegetation of this type consists of a mosaic of patterns, apparently associated with local variations in soil moisture, light intensity, and aspect. *Ledum groenlandicum* is common on all but the driest soils, usually associated with *Vaccinium vitis-idaea* ssp. *minor* and, less commonly, *Mertensia paniculata* and *Chamaenerion angustifolium*. In damp, shaded localities *Geocaulon lividum*, *Equisetum sylvaticum*, and *Petasites palmatus* are

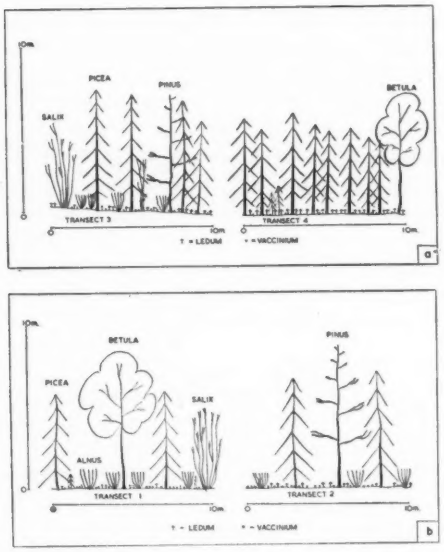


FIG. 5, *a* and *b*. Four profile diagrams of the closed mixed spruce/willow/alder forest. The upper transects, Nos. 3 and 4, show the transition zone to adjacent muskeg with an increased proportion of *Picea mariana* and *Ledum groenlandicum*. Transects 1 and 2 were recorded on a well-drained upper slope. The species are as indicated in Fig. 4.

frequent, while *Achillea millefolium*, *Chamaepericlymenum canadensis*, *Arctostaphylos uva-ursi* var. *adenotricha*, and *Linnaea borealis* ssp. *americana* are confined to gently sloping, drier substrata. In damp, humous, and shaded sites, *Lycopodium complanatum*, *L. annotinum* var. *pungens*, and *Mitella nuda* are locally frequent. Under shrubs of alder there is a characteristic assemblage of mosses of which *Pleurozium schreberi*, *Hylocomium splendens*, *Dicranum rugosum*, and *D. bergeri* are common members. In depressions where soil moisture accumulates *Sphagnum capillaceum* and *Aulacomnium palustre* are common. In Table III the results of a quadrat analysis indicate the structure and floristic composition of this community.

As in the case of the spruce forest, there is a transition from this closed spruce/willow/alder type to the muskeg and peat bog communities which occupy the hollows and small valleys which are common throughout this terrain.

The Open Pine/Birch/Willow Type

This type is a relatively open forest, dominated by *Pinus banksiana*. *Betula papyrifera* var. *neoalaskana* is frequent in the pine stratum, and there is a discontinuous tall-shrub stratum, consisting solely of species of *Salix*. The shrub layer is made up of more or less dense stands of *Alnus crispa*, often associated with young trees of *Picea mariana*. Two examples of this type will be described in detail as they illustrate the variations which are found

TABLE III

THE CLOSED SPRUCE/WILLOW/ALDER MIXED FOREST

Species	Quadrats (10 × 5 m.)						Presence class (7 sites)
	1	2	3	4	5	6	
Picea mariana	6(6–10 m.) 4(2–4 m.)	13(4–8 m.)	9(4–7 m.)	4(6–8 m.) 7(4–6 m.)	10(6–10 m.)	14(5–7 m.)	5
Pinus banksiana	–	1(7 m.)	1(9 m.)	1(10 m.)	–	1(9 m.)	5
Betula papyrifera var. *neoalaskana*	–	1(4 m.)	–	1(5 m.)	1(4 m.)	–	5
Salix bebbiana var. *perrostrata*	1(5 m.)	1(4 m.)	6(1 m.)	10(1–3 m.)	3(2–4 m.)	9(1 m.)	5
Picea mariana	6(1 m.)	–	7(1 m.)	14(1–2 m.)	15(1 m.)	3(1 m.)	5
Alnus crispa	9(1 m.)	11(1 m.)					
Ledum groenlandicum	d	a	d	f	a	f	5
Equisetum sylvaticum var. *pauciramosum*	–	o	o	o	o	o	5
Lycopodium annotinum var. *pungens*	–	–	–	–	–	r	3
L. complanatum	–	o	–	o	–	–	2
Grocaulon lividum	r	r	–	o	–	–	4
Mitella nuda	–	–	–	–	r	–	4
Ribes hudsonianum	–	–	o	–	o	o	3
R. triste	–	–	–	–	r	r	2
Rubus acaulis	lf	–	–	–	–	o	2
Rosa acicularis	o	o	–	r	–	o	5
Chamaenerion angustifolium	o	o	–	–	o	o	3
Chamaepericlymenum canadense	o	–	–	–	–	o	3
Arctostaphylos uva-ursi var. *adenotricha*	a	–	r	o	a	r	2
Vaccinium vitis-idaea ssp. *minor*	–	r	–	a	–	r	5
V. uliginosum	–	r	–	–	–	r	1
Meriensia paniculata	–	r	o	o	o	o	5
Linnaea borealis ssp. *americana*	–	r	–	–	–	o	4
Achillea millefolium	o	–	–	–	o	r	3
Petasites palmatus	o	o	r	–	o	r	5
Sphagnum capillaceum	o	–	r	o	–	–	3
Polytrichum juniperinum var. *alpestre*	–	–	r	o	–	–	4
Ceratodon purpureus	o	r	–	–	o	–	2
Dicranum rugosum	–	–	r	o	–	o	5
D. bergeri	–	lf	–	–	o	–	4
Aulacomnium palustre	o	lf	–	o	o	–	4
Pohlia nutans	r	–	la	o	o	o	3
Hylocomium splendens	–	lf	–	–	r	r	5
Ptilium crista-castrensis	o	lf	o	o	r	–	3
Pleurozium schreberi	–	lf	f	o	f	f	5
Ptilidium ciliare	–	f	o	f	–	–	3
Peltigera aphthosa	o	f	r	–	–	o	5
P. malacea	o	–	–	–	–	–	3
Stereocaulon paschale	r	–	r	o	–	–	3
Cladonia glauca	o	lf	r	–	o	o	2
C. mitis							

within the general structural composition outlined above. The first site is located at Station 1, Fig. 2. It consists of an open forest dominated by *Pinus banksiana* with occasional trees of *Betula papyrifera* var. *neoalaskana*. The tall-shrub layer consists of scattered individuals of *Salix pellita;* young trees of black spruce occur locally, and *Alnus crispa* is present in local abundance. The ground vegetation is dominated by *Ledum groenlandicum*, *Vaccinium vitis-idaea* ssp. *minor*, and *V. myrtilloides*, and by a locally continuous mat of lichens. The structure and floristic composition of this type are summarized in Table IV.

The soil of this site is a heavy clay. The surface of the ground is covered in part by many fallen tree trunks, mainly of pine and black spruce, indicating the occurrence of a forest fire in the recent past.

A detailed study of the forest vegetation at Station 1 (Fig. 2), based on field observations and the scrutiny of aerial photographs, yields information of interest. The area which is covered with vegetation of this open pine forest type is clearly defined and ends abruptly at its southwestern margin where a forest type predominates which is identical in all respects with the closed spruce forest type. Further, it is significant that the large island in Tod Lake opposite Station 1 is covered entirely with forest of the closed spruce forest type. The seral implications of this pattern of vegetation will be discussed later, but it should be pointed out here that the boundary between the two vegetation types appears to represent the limit reached by a forest fire which destroyed the previous forest of the area which is now covered by open pine forest. Therefore it is suggested that the open pine forest type represents a stage in the development of vegetation which has resulted from the destruction at least in part, of an earlier forest of the closed spruce type. Not unexpectedly the island in Tod Lake would appear to have escaped the effects of fire.

TABLE IV

THE OPEN SERAL PINEWOOD

Species	Quadrats (10 × 5 m.)						Presence class (4 sites)
	29	30	31	32	33	34	
Pinus banksiana	1(5 m.)	3(3–6 m.)	1(7 m.)	4(3–6 m.)	2(4–7 m.)	3(6 m.)	5
Betula papyrifera var. *neoalaskana*	1(6 m.)	–	1(7 m.)	–	–	–	5
Salix pellita	–	–	1(2 m.)	–	1(2 m.)	–	3
Picea mariana	1(3 m.)	–	–	1(4 m.)	1(2 m.)	–	5
Lycopodium annotinum	f	o	–	o	–	r	4
L. complanatum	r	o	–	–	o	r	4
L. obscurum	–	–	–	–	r	–	2
Juniperus communis var. *depressa*	–	–	–	r	–	–	1
Calamagrostis lapponica var. *nearctica*	o	–	–	o	–	o	4
Alnus crispa	–	–	–	o	–	o	4
Chamaecrion angustifolium	o	o	–	–	f	o	4
Chamaepericlymenum canadense	–	–	lf	–	o	–	2
Ledum groenlandicum	la	a	a	f	la	a	5
Vaccinium vitis idaea ssp. *minor*	f	o	f	f	la	f	5
V. myrtilloides	–	f	f	la	f	o	5
Cladonia coccifera	–	–	o	–	–	f	3
C. crispata var. *virgata*	–	–	–	r	–	r	2
C. deformis	la	f	o	–	–	o	4
C. gracilis var. *dilatata*	–	–	o	–	–	r	3
C. rangiferina	f	o	f	–	o	f	5

Field reconnaisance and the examination of aerial photographs reveal that this open pinewood with birch and willow is widespread in the area and varied in structure. One further example, of somewhat different structure, has been selected for detailed description; it is located at Station 11 (Fig. 3). This forest occurs extensively on gently sloping, undulating clay drift plains in the region immediately north of MacBride Lake. It is dominated by *Pinus banksiana;* throughout the forest are occasional trees of *Betula papyrifera* var. *neoalaskana*, and there is a discontinuous secondary tree stratum composed of *Salix bebbiana* var. *perrostrata*. *Alnus crispa* is abundant, forming a distinct shrub layer at about 1 m. The major physiognomic features of the vegetation are shown in Fig. 4*b* and Fig. 10, and the floristic composition is summarized in Table V. The differences between this type and the open pine forest described above from Tod Lake is clear from a comparison of Tables IV and V. The type being described at present shows a greater density of pines and the presence of a well developed shrub (alder) layer, both of which would appear to render conditions within the forest more mesic than in the more open forest. The ground vegetation of the former contains a higher proportion of mesophytic herbs and cryptogams than the latter.

It was noted in the field, and extensively confirmed by subsequent study of aerial photographs, that in certain areas north of MacBride Lake these pine stands were confined to the upper slopes and summits of this undulating terrain. In the wetter hollows and depressions were muskeg and bog communities, while narrow zones of the middle slopes and drier depressions bore open, almost

TABLE V

SERAL PINE FOREST

Species	Quadrats (10 × 5 m.)				Presence class (5 sites)
	1	2	3	4	
Pinus banksiana	4(5–7 m.)	7(5–7 m.)	5(5–7 m.)	6(6–7 m.)	5
Betula papyrifera var. *neoalaskana*	1(6 m.)	–	1(5 m.)	–	5
Salix bebbiana var. *perrostrata*	3(3–5 m.)	2(4 m.)	2(3–5 m.)	1(4 m.)	5
Alnus crispa (at 1–1½ m.)	d	a	d	a	5
Equisetum sylvaticum var. *pauciramosum*	r	–	–	o	3
Carex concinna	–	r	–	–	1
Ribes glandulosum	f	a	–	o	5
Rubus idaeus var. *strigosus*	o	o	–	f	4
R. pubescens	–	–	f	o	4
Rosa acicularis	f	o	f	f	5
*Fragaria vesca**	f	o	–	f	5
Chamaenerion angustifolium	o	r	–	r	4
Chamaepericlymenum canadense	–	–	o	–	2
Ramischia secunda	–	–	o	–	2
Pyrola asarifolia	–	o	–	–	3
Ledum groenlandicum	la	o	f	f	5
Vaccinium vitis-idaea ssp. *minor*	–	o	f	o	5
Mertensia paniculata	o	o	f	o	5
Linnaea borealis ssp. *americana*	f	f	o	f	5
Pelasites palmatus	o	–	–	–	3
Bryum cuspidatum	o	o	f	o	5
Eurhynchium diversifolium	–	lf	o	o	4
Peltigera aphthosa	–	o	–	o	4
P. canina var. *rufescens*	–	o	–	o	4
Cladonia rangiferina	–	o	–	–	3

**Used throughout in sensu lato.*

treeless tracts of "heath" vegetation. This unusual community was noted consistently fringing the lower limits of the pine forest; the only phanerophytic growth was scattered clumps of *Salix bebbiana* var. *perrostrata* and *S. arbusculoides*, occasional young trees of *Picea mariana*, and in one locality at Station 11 (Fig. 3) a few trees of *Populus balsamifera*. The ground vegetation is somewhat atypical for the area, consisting of a closed sward of prostrate plants, various herbs, and a discontinuous mat of fruticose and thalloid lichens. The general aspect of the vegetation is that of a heath, though the only ericaceous representative is *Arctostaphylos uva-ursi* var. *adenotricha*. Some consideration will be given to this community in the discussion at the end of this paper. Using the Braun-Blanquet (3, p. 32) scale, cover values of the component species of this community were estimated for six randomly selected 1 m. square quadrats. These data are summarized in Table VI.

Several forests in the region of study showed physiognomic differences from the main types which have been described above; some were intermediate between the closed spruce forest and the mixed spruce/willow/alder type, and others were intermediate between the latter and the open pine forest types. For example, a traverse of 4 km. due west from Station 13 (Fig. 3) revealed the following series of intergrading types (the stratum dominants are indicated): a birch/*Salix bebbiana* var. *perrostrata* scrub; a closed mixed spruce/alder forest; a semiopen pine/alder forest; and a closed spruce/willow/alder mixed

TABLE VI

COVER VALUES FOR THE OPEN "HEATH" COMMUNITY

Species	Quadrats (1 m. square)					
	1	2	3	4	5	6
	Cover values					
Fragaria vesca	2	1	1	1	1	2
Arctostaphylos uva-ursi var. *adenotricha*	1	3	2	1	2	1
Peltigera canina var. *rufescens*	3	2	1	2	2	3
Carex deflexa	1	+	1	2	+	+
Oryzopsis pungens	+	+	+	+	+	+
Rosa acicularis	+	+	+	−	+	+
Chamaenerion angustifolium	1	+	+	+	+	+
Mertensia paniculata	+	+	+	+	−	+
Petasites palmatus	+	+	−	+	−	+
Rubus pubescens	1	+	1	+	1	1
Solidago multiradiata	1	1	−	+	1	−
Achillea millefolium	+	+	+	+	+	+
Bare soil	1	+	1	1	1	1
Cladonia rangiferina	−	+	+	1	1	+
C. crispata var. *virgata*	−	1	+	+	−	+
Botrychium lunaria	−	−	+	−	−	−
Chamaepericlymenum canadense	−	−	1	−	−	−
Senecio pauperculus var. *flavovirens*	−	−	+	−	−	−
Linnaea borealis ssp. *americana*	−	−	+	−	1	−
Mitella nuda	−	−	−	+	−	−
Stereocaulon paschale	−	−	−	+	−	+
Aquilegia brevistyla	−	−	−	−	−	+

forest. Over this traverse no apparent changes in habitat were observed, and it seems clear that these types, together with the main types described above, are related serally to the closed black spruce forest type.

In the region of Tod Lake (Fig. 2) an early phase of the regeneration of vegetation on mesic sites after fire is present, represented by two stands. One of these (at Station 11) is dominated by young trees of *Betula papyrifera* var. *neoalaskana* of 2–3 m. height and 4–7 cm. diameter at 20 cm. from ground level. They form a dense stand, associated locally with young trees of *Pinus banksiana* and, very rarely, individuals of *Picea mariana*. *Alnus crispa* occurs sporadically and *Ledum groenlandicum* and *Vaccinium vitis-idaea* ssp. *minor* are common in the ground vegetation; there is a very shallow upper layer of humus in the soil profile. The other stand (at Station 8) consists of a mixed community of young individuals of birch, pine, and spruce, forming a discontinuous stratum at 1–2 m.; it is an open, scrub type of vegetation with dominance shared by birch and spruce. The upper humous horizons of the soil profile are well preserved, and locally there are patches of mature spruce which appear to have survived the effects of the fire; these facts indicate that the fire was of a relatively superficial nature. The ground vegetation is varied and relatively rich in species, bearing close resemblances to that of the mixed spruce/willow/alder forests which have been described above.

It would appear that these two stands represent two of the possible sequences of recolonization after an area of pure black spruce or a closely related less mature forest has been burned; and their different structures indicate that the nature of the subseral communities depends in part on the degree of destruction of both the original forest and the upper, organic component of the substratum. At Station 11 the effects of the fire have been severe and little humus remains; here birch has spread rapidly and formed a dense seral stand. At Station 8 all the evidence indicates that the fire effected no more than the incomplete destruction of the trees while little of the ground vegetation and upper soil horizons were destroyed. In this latter case a more rapid and direct return to the original forest would result.

It is clear that, for this area, the closed spruce forest represents the most advanced stage of forest vegetation on moderately drained, mineral substrata and that these several forest types which have been described, occurring on similar substrata, are phases in the sequence of changes which is precipitated by the destructive effects of fire. The relic stumps of white birch in the pure spruce forests, the abrupt transitions between open pine forest types and closed spruce forests with no associated site changes, and the preponderance of young spruce trees in many of these mixed forest types, are clear evidence that there is a tendency for the vegetation to develop towards the black spruce forest type. A multiplicity of factors must determine both the rate of development after fire and the nature of the phases which culminate in a stable forest. The degree of destruction of the vegetation at the time of the fire, and the recurrence of fires, will determine the structure and composition of the recolonizing vegetation. It would appear that *Pinus banksiana*, *Betula*

papyrifera var. *neoalaskana*, and several shrubs, by their more rapid rate of initial growth (cf. Moss (33, p. 229)), assume the roles of stratum dominants in areas which have been cleared by fire. As a closed forest develops, coincident with the development of a more mature substratum, conditions become favourable for the growth of black spruce, which ultimately replaces these various stratum dominants, intolerant of shade, resulting in the pure, closed spruce forest. The effect of decreased illumination would appear to be the elimination of herbs in the ground layers which are replaced by a dense carpet of mosses.

The Pine Forests

In this area there are two physiographic types which bear mature pine forests; the vegetation of these types will be described.

Outcrop Ridges

High outcrop ridges, generally of pre-Cambrian, intrusive granites, are a common feature both of the area of study and of the Canadian Shield in general. On the summits of these ridges of exposed rocks with little weathered or drift soil an open pine forest develops. An extensive forest of this type on the large granite outcrop plateau at Station 14 (Fig. 3) was selected for intensive study.

The trees of *Pinus banksiana* occur in shallow crevices and depressions in the rocks and seldom attain more than 8 m. in height. On the flanks of the ridge, where mineral soil accumulates in depressions and hollows, *Picea mariana* is found. On the summit at this site a discontinuous and very sparse tall-shrub stratum was recorded, represented by individuals of *Salix pyrifolia* and *Betula papyrifera* var. *neoalaskana*. The nature of the ground vegetation depends closely on the type of substratum. On the exposed outcrop surfaces a dense carpet of saxicolous lichens is present while the soil-filled interstices bear a varied assemblage of both cryptogamic and phanerogamic plants. In drier, humous-filled crevices and shallow hollows *Vaccinium myrtilloides* is dominant, and *V. vitis-idaea* ssp. *minor* and *Empetrum hermaphroditum* are locally abundant. In smaller cracks and crevices *Corydalis sempervirens*, *Carex deflexa*, and *C. brunnescens* are found associated with various lichens. Occasionally there are deep hollows in the ridge plateau where considerable organic soil has developed under conditions of impeded drainage; in such a site the following species were recorded: *Ledum groenlandicum*, *Picea mariana* (small trees), *Eriophorum spissum*, *Aulacomnium palustre*, *Sphagnum capillaceum*, and *Polytrichum commune*.

There is a narrow ecotone between the ridge pine forests and the black spruce communities on the marginal terrain of drift soil; this zone is marked by the extension of the respective dominants of each type into the two communities, depending on the disposition of rock and mineral soil. The general aspect of this community is illustrated by Fig. 9, and the quadrat analysis which is presented in Table VII indicates the spatial relationships of the component species.

TABLE VII

THE OUTCROP RIDGE PINE FOREST

Species	Quadrats (10 × 5 m.)				Presence class (7 sites)
	13	13a	14	15	
Pinus banksiana	3(6 m.)	5(4–6 m.)	4(6 m.)	6(4–6 m.)	5
Picea mariana	3(1 m.)	2(1 m.)	–	1(2 m.)	5
Betula papyrifera var. *neoalaskana*	–	–	1(2 m.)	–	3
Salix pyrifolia	–	1(1 m.)	–	1(2 m.)	2
Carex deflexa	o	r	–	f	4
C. brunnescens	o	f	o	f	5
Corydalis sempervirens	–	r	o	–	5
Empetrum hermaphroditum	o	f	o	o	3
Vaccinium myrtilloides	ld	a	a	la	5
V. vitis-idaea ssp. *minor*	f	f	f	o	5
*Andreaea rupestris**	–	o	–	–	3
Polytrichum juniperinum var. *alpestre*	o	–	–	o	5
*Actinogyra muehlenbergii**	a	o	f	f	5
*Parmelia centrifuga**	a	f	f	a	5
*P. stenophylla**	o	f	f	o	5
*Cetraria hepatizon**	f	f	f	f	5
Cladonia alpestris	o	o	f	–	4
C. rangiferina	a	f	f	f	5

**Exclusively saxicolous species.*

Within the area of study a high outcrop ridge of sedimentary greenstone rock provided an interesting comparison with the more common granite ridge community described above. This ridge is on the south shore of MacBride Lake, at Station 3 (Fig. 3). It appears that there is a greater amount of soil on the greenstone ridges than on the granite since the former rock weathers rather more readily than the latter, and this difference is expressed in a different dispersion of community types. In both cases there is a well developed mat of lichens on the exposed outcrop surfaces, and the shallow interstices and crevices bear trees of *Pinus banksiana* with similar associated shrubs and herbs on both types. However, owing to the greater soil development on the greenstone, the spruce forest of the flanks advances on to the ridge, restricting the area of continuous pine forest. And there is a greater range of edaphic conditions on the greenstone ridge, evinced by the presence of pine forest, saxicolous lichen communities, units of spruce-dominated forest, and pockets of spruce muskeg, all within an area of less than 50 sq. m. in certain instances.

Perhaps the most effective method of describing this vegetation is by mapped quadrats. Three of these, from Station 3 (Fig. 3), are shown in Fig. 6. In Quadrat 24 the rocks are covered with a diversity of lichens, chiefly *Actinogyra muehlenbergii*, *Parmelia centrifuga*, *Stereocaulon paschale*, *Cetraria hepatizon*, along with *Andreaea rupestris*. Immediately abutting the outcrops is a thin humous soil bearing a mat of cryptogamic vegetation, chiefly composed of

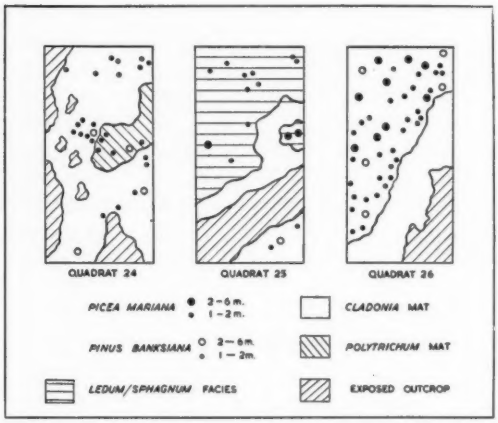


FIG. 6. Three quadrat charts from an outcrop ridge of greenstone at MacBride Lake. Detailed descriptions of the vegetation of the quadrats is available in the text.

Cladonia coccifera, *C. crispata* var. *virgata*, *C. rangiferina*, and *Polytrichum juniperinum* var. *alpestre*. The depression is dominated by *Polytrichum commune* associated with *Sphagnum warnstorfianum*, *Dicranum rugosum*, *Oxycoccus microcarpus*, *Ledum groenlandicum*, and *Rubus chamaemorus*. There are several young trees of black spruce, and jack pine is confined to the drier sites. Quadrat 25 illustrates the vegetation pattern on a peat area developed under the conditions of impeded drainage over a flat outcrop surface. The ground vegetation under *Picea mariana* consists of a dense, hummocked carpet of *Sphagnum warnstorfianum* dominated by *Ledum groenlandicum* with *Oxycoccus microcarpus* and *Rubus chamaemorus;* that is, a local facies of muskeg. Adjacent to the narrow lichen covered area of outcrop is a thin humous layer with a typical assemblage of *Cladonia* species. One plant of *Alnus crispa* was recorded in this quadrat, in the lichen mat abutting on the muskeg area. Quadrat 26 shows the transitional zone between the outcrop ridge and the soil-covered slope sites, the latter spruce-dominated. The vegetation of the outcrop in this quadrat included, in humous-filled crevices, *Potentilla tridentata*, *Agrostis scabra*, *Chamaenerion angustifolium*, and *Corydalis sempervirens*. In the lichen mat *Vaccinium myrtilloides* and *V. vitis-idaea* ssp. *minor* were recorded. In the deeper soil under a closed spruce/pine canopy were recorded *Alnus crispa*, *Vaccinium vitis-idaea* ssp. *minor*, *Pleurozium schreberi*, *Dicranum fuscescens*, and *Polytrichum juniperinum* var. *alpestre*.

This comparison between the summit communities of granite and greenstone ridges would appear to indicate that the operative factor which determines the relative proportions of pine and spruce, in this particular xeric habitat, is edaphic. On the granite ridges where there is slow weathering and limited soil development, conditions appear to be inimical to spruce, and pine maintains dominance. However, on the greenstone ridge the proportion of habitats

which are beyond the range of tolerance of spruce is less and it is common there, often sharing dominance with pine on the summits; and in certain summit sites it assumes complete dominance. However, in the area of this study, and in the Canadian Shield generally (within the distribution area of jack pine), the greater number of outcrop ridges are of intrusive granitic rocks, and it would appear to be justified to recognize the pine community here as the mature or stable type.

On Areas of Sand

On outwash plains and eskers which show no evidence of recent disturbance the dominant vegetation is a closed pine forest. A detailed study was made of a sand plain forest adjacent to a high outcrop ridge at the south end of Tod Lake at Station 9 (Fig. 2). This area is covered by a dense, pine-dominated forest with a uniform vegetation of few species. The substratum consists of a fine-grained sand; there is an upper horizon of black, fibrous humous (2–4 cm.), a gray-white, humous-stained layer (10–15 cm.) with bleached sand grains, and a deep horizon at 20 cm. of reddish-brown sand. The pines are 12–16 m. tall with diameters at 1 m. from the ground level of 14–20 cm. They are well grown trees with straight trunks. There is no

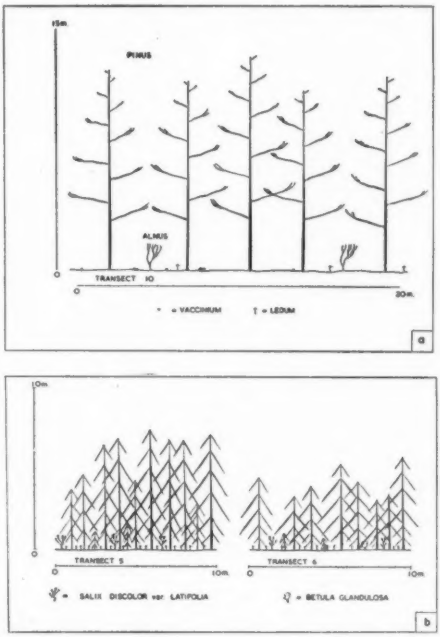


FIG. 7*a*. A profile diagram of the jack pine forest on a sand plain at Tod Lake. The species noted are *Pinus banksiana*, *Alnus crispa*, and *Vaccinium vitis-idaea* ssp. *minor*.

FIG. 7*b*. Profile diagrams of muskeg vegetation, dominated by *Picea mariana*, described in detail in the text and summarized in Table VIII.

subsidiary tree or shrub stratum, but *Picea mariana* and *Betula papyrifera* var. *neoalaskana* (rare) occur infrequently throughout the forest. The ground vegetation is a closed sward of lichens, mosses, and prostrate shrubs. Fig 7*a* shows a profile diagram of this community. The following list was compiled at Station 9:

Vaccinium vitis-idaea ssp. *minor*	– la	*Pyrola asarifolia*	– o
Arctostaphylos uva-ursi var. *adenotricha*	– la, o	*P. virens*	– r
Ledum groenlandicum	– r	*Pleurozium schreberi*	– o
Geocaulon lividum	– o, lf	*Polytrichum juniperinum* var. *alpestre*	– o
Maianthemum canadense	– o	*Cladonia rangiferina*	– o, lf
Chamaepericlymenum canadense	– o		

On the north side of MacBride Lake at Station 1 (Fig. 3) there is a small esker which runs in a north/south direction. The abundance of old fallen pine trunks on the summit and flanks indicates clearly that the area has been burned, and the structure of the present vegetation indicates that it is a seral phase. The vegetation is an open pinewood, with *Betula papyrifera* var. *neoalaskana* assuming very local dominance. Widespread in the community is *Salix bebbiana* var. *perrostrata* which forms tall shrubs. Occasional small trees of *Populus tremuloides* were recorded on the flanks of the esker. The following list of plants was compiled at this station:

Upper strata	
Pinus banksiana	– d (4–6 m.)
Salix bebbiana var. *perrostrata*	– f
Populus tremuloides	– o
Alnus crispa	– o
Betula papyrifera var. *papyrifera*	– o, ld
Ground vegetation	
Arctostaphylos uva-ursi var. *adenotricha*	– f, ld
Vaccinium vitis-idaea ssp. *minor*	– a
Rosa acicularis	– o
Viburnum edule	– o
Picea mariana	– o
Larix laricina	– r (in damp hollows)
Ledum groenlandicum	– r (in damp hollows)
Juniperus communis var. *depressa*	– lf (on slopes)
Aquilegia brevistyla	– r
Chamaenerion angustifolium	– f
Fragaria vesca	– o
Chamaepericlymenum canadense	– o
Pyrola asarifolia	– o
Mertensia paniculata	– o
Aster ciliolatus	– r
Solidago multiradiata	– r
Achillea millefolium	– r
Petasites palmatus	– o
Stereocaulon paschale	– o
Cladonia rangiferina	– f
C. glauca	– f
C. crispata var. *virgata*	– o
Peltigera malacea	– f
P. canina var. *rufescens*	– r

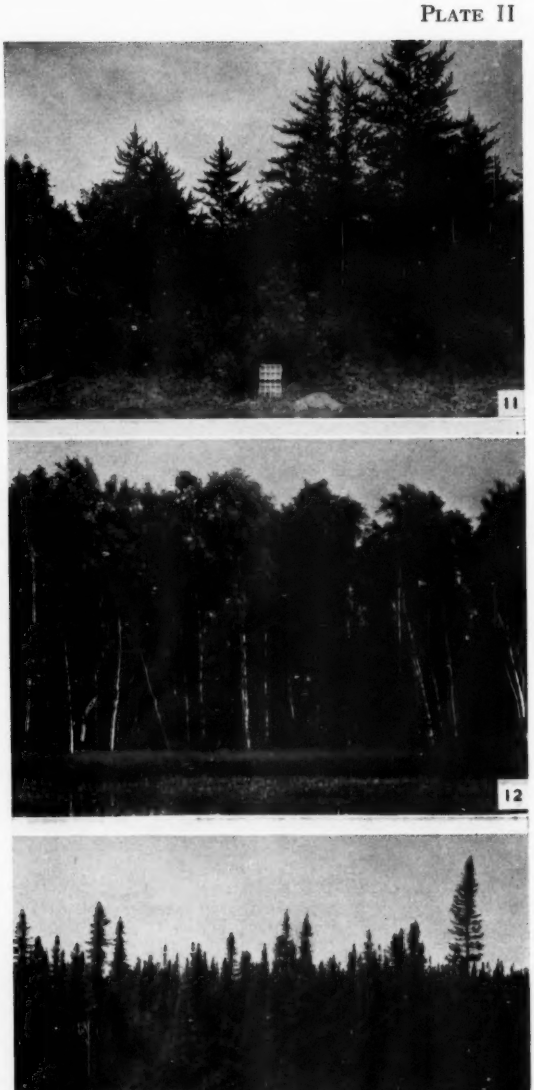
It is of interest here to note that there are close physiognomic and floristic resemblances between the ground vegetation of this open, seral pinewood and that of the tracts of heath vegetation in pine stands at Station 11 (Fig. 3), described above.

FIG. 8. A general view of the mature closed black spruce forest on a till ridge. In the foreground the shore line fringe community of willows and birch, referred to in the text, is visible.

FIG. 9. A view from the edge of a large granite outcrop plateau showing the ecotone between the open jack pine forest of the summit (foreground trees) and the black spruce forest of the ridge slopes. In the foreground the lichen-covered rock surface is seen.

FIG. 10. The interior of the closed black spruce forest showing an old stump of *Betula*, held erect by the resistant outer cylinder of bark.

PLATE II



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White Spruce Communities

Throughout the area of this study *Picea glauca* is of sporadic occurrence, never forming more than local segments of forest. Only three of these were examined in detail, and only five were recorded for the entire area.

One small stand occurs on the north shore of MacBride Lake at Station 2 (Fig. 3). It is confined to a steep shore line slope of 35°–40° inclination and approximately 35 m. in height. This slope has a southern aspect and consists of a dry, sandy loam soil of fine texture. Confined to this area, which extends along the lake shore for no more than $\frac{1}{4}$ km., is a forest which differs in every respect from the typical closed black spruce forest which is contiguous with it inland and on both sides. It consists of widely spaced individuals of *Picea glauca* of large stature, the tallest reaching 30 m. with a trunk diameter of 60 cm. at 1 m. from ground level. Scattered between the white spruces are several old trees of *Populus tremuloides* and *P. balsamifera*, both about 10–15 m. in height. One or two mature trees of *Picea mariana* and *Betula papyrifera* var. *neoalaskana* are associated with the poplars and white spruces. The ground flora is as highly distinctive as the tree strata; it includes several species which are otherwise found only in exposed, dry, usually south-facing habitats, such as sunny outcrops and shore lines. The following were recorded:

Juniperus communis var. depressa	– ld, a	*Viburnum edulea*	– o
Arctostaphylos uva-ursi var. *adenotricha*	– lf, a	*Cornus stolonifer*	– o
Vaccinium vitis-idaea ssp. *minor*	– a	*Aralia nudicaulis*	– o
Chamaenerion angustifolium	– f	*Pyrola asarifolia*	– o
Alnus crispa	– o	*Mertensia paniculata*	– o
Poa glauca	– o	*Shepherdia canadensis*	– r
Geocaulon lividum	– o	*Ramischia secunda*	– r
Rosa acicularis	– o	*Rhytidium rugosum*	– ld, a
Fragaria vesca	– o	*Cladonia mitis*	– a

Behind this slope, on the moderately drained soil of the plateau, the typical closed black spruce forest dominates; there is no evidence that this transition can be associated with any factor other than the aspect and drainage of the substratum.

The second white spruce community which it was possible to study in detail is somewhat more fragmentary and discontinuous than that which has just been described. It is found on the northwest shore of Tod Lake, extending between Stations 2 and 4 (Fig. 2). As at MacBride Lake, this community is found on a steep shore line slope with a southern aspect. Here however, the exceptional, relatively high relief extends along the entire northern shore

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FIG. 11. A view of an open seral pine forest showing the three main phanerophytic strata: a continuous alder layer at 1 m., a discontinuous willow layer at 2–3 m., and an open upper stratum of jack pine and white birch.

FIG. 12. A view of the birch-dominated marginal peat ridge at MacBride Lake, showing the aquatic lake vegetation (foreground), the narrow zone of sedge-dominated bog, and the closed stand of *Betula papyrifera* var. *neoalaskana* of the ridge.

FIG. 13. A view of the abrupt transition between peat bog (foreground) and muskeg; the uneven, stunted nature of the spruce stand is illustrated. The tall tree on the right hand side is about 7 m. in height. The vegetation of the peat bog zone which is shown consists of a ground cover of *Sphagnum* (not visible) bearing various species of *Carex* and occasional shrubs of *Betula glandulosa*.

of Tod Lake; this is due to the presence of an uncommon feature of geology—the complex of faulting to which reference has already been made. This slope consists of a diversity of edaphic types, including small cliffs and crevices, sloping outcrops, loose detritus, and pockets of deep, well drained soil. On the lower slopes occur widely spaced individuals of *Picea glauca*, 15–20 m. tall, associated with occasional individuals of *P. mariana* and *Betula papyrifera* var. *neoalaskana*. The ground vegetation immediately associated with these spruces, on well developed, dry, loamy soil yielded the following list of plants: *Ribes oxyacanthoides*, *Amelanchier alnifolia*, *Prunus pensylvanica*, *Aralia nudicaulis*, *Shepherdia canadensis*, *Viburnum edule*, *Chamaenerion angustifolium*, and *Linnaea borealis* ssp. *americana*.

The summit vegetation consists of a young forest of white birch, aspen, and jack pine while the inland slope, with a northwest aspect, is dominated by a mature, closed black spruce forest. Locally on the southwest shore the white spruce community is replaced by dense stands of young birch, and this and the nature of the summit vegetation would indicate that there has been local forest burning on the ridge.

It is clear that, by its physiography and geology, this ridge is atypical and of particular interest; the vegetation of certain of its specialized habitats will be described in detail in a later section; the floristic interest of this locality is referred to elsewhere (Ritchie (46)).

One stand of mixed forest containing *Picea glauca* was observed which bore certain structural and floristic similarities to the rich mixed forests which are more common in southern areas of the boreal forest region of Manitoba. This stand is at Station 10 (Fig. 3), on the MacBride River, occupying an area of about 100 × 50 m. The substratum is of the alluvial type, consisting of a well developed, relatively rich black-earth soil. The stand is dominated by mature trees of *Populus balsamifera*, *P. tremuloides*, *Picea glauca*, and *Betula papyrifera*, forming a closed canopy at 20–25 m., the balsam poplar and spruce being 30–35 cm. in diameter at 1 m. from ground level. There is little tree stratification, the only subsidiary layer being represented by scattered individuals of *Salix bebbiana* var. *perrostrata*. There is a conspicuous shrub layer at 40–100 cm. consisting of *Alnus crispa*, *Viburnum edule*, *Cornus stolonifera*, *Rubus idaeus* var. *strigosus*, *Ribes oxyacanthoides*, and *Rosa acicularis*. The ground vegetation is made up of the following species, in approximate order of abundance: *Aralia nudicaulis*, *Linnaea borealis* ssp. *americana*, *Chamaepericlymenum canadensis*, *Calamagrostis canadensis*, *Brachythecium salebrosum*, *Eurhynchium diversifolium*, *Mertensia paniculata*, *Pyrola asarifolia* *Mitella nuda*, *Fragaria vesca*, *Petasites palmatus*, and *Chamaenerion angustifolium*. (As it was possible to spend only a short time examining this stand the above account of the vegetation is incomplete, including only the more common plants of the ground vegetation.) At no other place in the areas of study was a community of this kind observed.

One can conclude from these studies that, in this region, *Picea glauca* is confined to very local physiographic features, being associated on the one hand with well drained, light-textured mineral soils found on slopes with a southern aspect, and on the other with rich alluvial soils of river valleys; that is, only under optimum conditions of local climate and soil is white spruce represented in the vegetation of this area. And the types which occur correspond with the "upland mesophytic forest" and "flood plain forest" types of Raup (44).

A Local Birch Community

Perhaps the most unusual and apparently atypical unit of vegetation which was examined was represented by five stands of mature white birch forest, all in the vicinity of MacBride Lake, at Stations 6 and 9 (Fig. 3). The distinctive feature which is common to these stands is that they occur on raised domes or terraces of organic substratum, which on one side abut on outcrop and drift ridges and on the other side are bounded by various shallow arms and bays of MacBride Lake. Since the physiography and vegetation of these communities is completely uniform, one stand (at Station 6) will be described in detail.

This community is readily distinguished, both in the field and on aerial photographs, by the uniform cover of white birch crowns (Fig. 12), and it has been possible to locate on the photographs several additional stands which were not visited; these are, without exception, confined to the same drainage system as those which were observed in the field. The stand is unstratified, being made up of a dense canopy of *Betula papyrifera* var. *neoalaskana*, which is associated with occasional slender trees of *Salix petiolaris*. The birches are 15–20 m. tall and 20–25 cm. in diameter at 1 m. from the ground. They form an uneven-aged community with considerable evidence of regeneration. A detailed examination of the ground vegetation yielded the following list of plants, arranged in order of relative abundance:

Calamagrostis canadensis	– f, ld	*Mitella nuda*	– r
Rubus idaeus var. *strigosus*	– f	*Scutellaria epilobiifolia*	– r
Ribes glandulosum	– f	*Actaea rubra*	– r
Urtica gracilis	– lf	*Pyrola asarifolia*	– r
Chamaenerion angustifolium	– o	*Ramischia secunda*	– r
Viburnum edule	– o	*Rosa acicularis*	– r
Equisetum sylvaticum	– o	*Brachythecium salebrosum*	– f
Mertensia paniculata	– o	*Pohlia nutans*	– o
Rubus pubescens	– o	*Hylocomium splendens*	– o
Geum perincisum	– r	*Cladonia gracilis*	– o (f)

In addition, the following epiphytes of white birch were noted; *Parmelia sulcata*, *P. olivacea*, and *Evernia mesomorpha*.

Locally, there is a zonation of vegetation between the open water of the lake and the birchwood. The aquatic vegetation of the lake, which seldom exceeds 1–2 m. in depth, consists of dense patches of *Nuphar variegatum*, associated with *Sagittaria cuneata*, *Drepanocladus sendtneri*, and *Sparganium angustifolium*. There is a marginal zone of vegetation of *Calla palustris*, *Carex vesicaria*, *Carex lasiocarpa* var. *americana*, *Cicuta mackenzieana*, and *Comarum*

palustre.* Behind this is a zone of bog vegetation, in which the following were recorded, arranged in approximate order of abundance: *Betula glandulosa*, *Chamaedaphne calyculata* var. *angustifolia*, *Salix pedicellaris* var. *hypoglauca*, *Sphagnum warnstorfianum*, *Camptothecium nitens*, *Vaccinium uliginosum*, *Sphagnum fuscum*, *Ledum groenlandicum*, *Andromeda polifolia*, *Smilacina trifolia*, *Triglochin maritima*, *Eriophorum angustifolium*, *Carex limosa*, *C. aquatilis*, *C. diandra*. Between this zone and the raised birchwood is a narrow transition with occasional trees of *Picea mariana* and *Larix laricina*, with *Alnus crispa* and *Betula glandulosa*. It would appear that this zonation of vegetation, including the birch community, is related solely to soil moisture, as there is no discontinuity of substratum type between the open lake and the dry forest soil.

One of the stands, at Station 9, shows an eroding margin, abutting onto a shallow bay of the lake in which there are local islands of *Calla palustris* with various associated bog plants. This vertical exposure of peat provides an excellent opportunity to establish the fact of the complete homogeneity of the organic substratum, since it reveals a profile of 4–5 m. of unstratified, fibrous, relatively dry organic material. In all localities the height of the ridge is 3–6 m. above the level of the adjacent lake surface. Within the communities there is no evidence that the birch is being replaced by any other trees, or that it has suffered any recent anthropogenic catastrophe. In one part of one stand there is some evidence of slight burning, but the few felled trees are of white birch and the regeneration is only by this tree. Thus it would appear that the peculiar edaphic properties of this dry organic substratum favour the establishment and maintenance of a birch forest.

As far as the present writer is aware there is no report in North American literature of a phenomenon which could be identified with this community on organic ridges. And in fact, by personal communication, it has been found impossible to date to find any botanist or other traveller in North America to whom this association of vegetation and substratum is familiar. As has been indicated above, these stands of birch were confined to one particular system of lakes and small rivers immediately to the south of MacBride Lake (Fig. 3). It would be inappropriate to discuss at length here the possible ways in which this unique formation has arisen. Suffice it to note that it is not possible to assign it to any of the categories of lake ridge phenomena which have been described for various regions in boreal and subarctic Canada by Tyrrell (56), Lewis, Dowding, and Moss (28), and Raup (39). It would appear that a considerable lowering in the level of the water of the drainage system to which these ridges are confined has exposed local peripheral deposits of peat which must have formed *in situ* at some previous time when the level of the water was considerably higher. The local persistence of these exposed peat bodies, and the subsequent improved drainage of the upper layers of organic material would appear to have favoured the entry and eventual dominance of white birch.

*= *Potentilla palustris*.

Muskeg and Peat Bog

Muskeg

Over the area of this investigation only minor variation in the structure and floristic composition of muskegs was observed. Thus the detailed description of one example will provide a representative account of the community. A large muskeg at Station 12 (Fig. 3) was examined in detail and will be described here. It forms a discrete zone in the flat, low depression between the surrounding ridges of closed spruce forests and the open peat bogs which occupy the center of the basin. The most characteristic features are the irregular nature of the surface and the open stand of poorly grown trees of *Picea mariana*, as illustrated in Fig. 13. The trees seldom exceed 7 m. in height, more commonly being about 5 m. There is a discontinuous shrub stratum at 1–2 m., made up of *Salix bebbiana* var. *perrostrata* and *Betula glandulosa*, with occasional individuals of *Salix pyrifolia*. There is a low-shrub stratum of which *Ledum groenlandicum*, *Kalmia polifolia*, and *Salix myrtillifolia* are common members. The ground vegetation consists entirely of a moss carpet bearing various herbs and prostrate shrubs, of which *Rubus chamaemorus*, *Smilacina trifolia*, *Vaccinium vitis-idaea* ssp. *minor*, and *Oxycoccus microcarpus* are the most characteristic. The chief moss species form hummocks throughout the muskeg—they are *Sphagnum capillaceum*, *S. fuscum*, and less commonly *Aulacomnium palustre* and *Camptothecium nitens*. In slightly drier sites, commonly directly under spruce trees, are *Pleurozium schreberi*, *Hylocomium splendens*, and *Dicranum bergeri*. Scattered in the surface of large hummocks of *Sphagnum capillaceum* are plants of *Pohlia sphagnicola*. The thalloid lichen *Peltigera aphthosa* is common in the drier weft-form moss carpets, usually associated with *Pleurozium schreberi*.

Table VIII summarizes the floristic composition and structure of the muskeg; the spatial relationships of the strata are depicted in Fig. 7*b*.

One muskeg which was examined (at Station 12, Fig. 3) revealed a local abundance of *Pinguicula villosa*, partly immersed in the surface of a large hummock of *Sphagnum fuscum;* in the same habitat *Ramischia secunda* var. *obtusata* is locally common. At a single locality at Tod Lake *Drosera rotundifolia* was recorded in local abundance along with *Rubus chamaemorus* and *Pinguicula villosa*. The following species were recorded from various muskegs, in wetter areas; they are more typical of open peat bogs: *Carex limosa*, *Myrica gale*, and *Andromeda polifolia;* also, somewhat exceptionally, *Empetrum hermaphroditum* and *Geocaulon lividum* were recorded from a muskeg at Station 1 (Fig. 2), growing in local areas of improved drainage.

As is indicated above, there is some floristic variation in muskeg communities but the general pattern of vegetation is constant. One exceptional example merits attention; it occurs at Station 3 (Fig. 2) and it borders a small lake inland from the northwest shore of Tod Lake. By criteria of substratum, physiognomy, and floristic composition this community would be assigned readily to the muskeg type of vegetation; however, there is here a remarkable abundance of *Pinus banksiana*, completely concomitant with *Picea mariana*

TABLE VIII

MUSKEG VEGETATION

Species	Quadrats (10 × 5 m.) 8	9	10	11	Presence class (10 sites)
Picea mariana	9(4–5 m.) 13(1–2 m.)	9(3–6 m.) 7(1–2 m.)	10(3–7 m.) 8(1–2 m.)	8(4–7 m.) 6(1–2 m.)	5
Salix discolor var. *latifolia*	1(1 m.)	2(1–2 m.)	1(1 m.)	2(1–2 m.)	5
S. pyrifolia	2(2 m.)	–	–	–	3
Betula glandulosa	f	o	f	o	5
Salix myrtillifolia	–	la	la	o	5
Ledum groenlandicum	la, f	a	a	f	5
Kalmia polifolia	o	–	o	–	4
Chamaedaphne calyculata var. *angustifolia*	–	–	–	r	3
Arctous rubra	o	o	–	o	2
Vaccinium uliginosum	lf	o	o	f	4
V. vitis-idaea ssp. *minor*	lf	–	la, f	f	5
Equisetum sylvaticum var. *pauciramosum*	f	o	–	o	3
Calamagrostis canadensis	–	r	–	–	2
Carex gynocrates	–	r	–	–	2
C. aquatilis	o	o	–	–	5
Smilacina trifolia	o	–	o	–	4
Rubus chamaemorus	f	o	f	f	5
Oxycoccus microcarpus	o	–	f	f	5
Petasites palmatus	r	–	–	o	3
Sphagnum capillaceum	d	ld	a	d	5
S. fuscum	a	o	f	f	5
Polytrichum juniperinum	–	o	–	–	3
Dicranum bergeri	lf	o	–	o	5
Aulacomnium palustre	f	f	f	f	5
Pohlia sphagnicola	r, lf	–	–	r, lf	2
Camptothecium nitens	o	la	o	lf	5
Pleurozium schreberi	o	f	lf	o	5
Hylocomium splendens	–	–	o	–	3
Peltigera aphthosa	lf	o	–	o	4
Cladonia glauca	lf	–	o	o	3

in deep wet *Sphagnum*, and in about the same abundance as the black spruce. The pine grows in all parts of the muskeg, including the edge of the lake where it is associated with *Myrica gale*, *Betula glandulosa*, *Chamaedaphne calyculata* var. *angustifolia*, and *Alnus crispa*. For this area, and generally, jack pine is not a typical member of muskeg communities and certainly seldom assumes codominance there with black spruce. As is often the case, an examination of the aerial photographs for this locality provides some clue to the explanation of this anomaly. On the east side of the muskeg, across the small lake, is a stand of closed black spruce forest; however, abutting the muskeg in all other directions is an extensive stand of open pinewood—the community which is summarized in Table IV above. There is clear evidence that the surrounding forest was destroyed by fire, at least in part, a few decades ago, and it is probable that the muskeg was also affected. In any case, it would appear that the presence and relative abundance of *Pinus* in this muskeg are determined

by the unusual preponderance of pines in the immediate vicinity; this has brought about an even balance between pine and spruce in the muskeg. Observations of other muskegs confirm this suggestion, since it was noted that muskeg is associated (spatially) more commonly with spruce forest than with pine forest.

Some reference was made above to the transitional zone between muskeg and spruce forest. This gradual ecotone is found where organic substrata abut upon mineral soils. It is marked by an extension into the periphery of the forest by typical bog and muskeg plants, such as *Rubus chamaemorus*, *Arctous rubra*,* *Vaccinium uliginosum*, *Sphagnum capillaceum*, and *Aulacomnium palustre*. Within this ecotone there is a discontinuous facies made up of a group of plants which are associated with neither typical spruce forest nor typical muskeg communities. They are more or less confined to a particular edaphic phenomenon which occurs at the junction of the organic and mineral soils; in such sites there are small, slightly raised mounds of mineral soil surrounded by wet *Sphagnum* peat. It appears that the local eutrophic soil conditions of these habitats are favourable to these plants; experience of the habitats of these plants in other areas and communities confirms this suggestion. In the wet marginal peat are found *Carex vaginata*, *C. concinna*, *Coptidium lapponicum*,† *Ranunculus abortivus* var. *acrolasius*, *R. purshii* (occasional), *Epilobium palustre*, and *Smilacina trifolia*, while on the drier mineral soil of the low mounds *Stellaria longifolia* and *Fragaria vesca* are common.

At Station 4 (Fig. 3) a slow-running stream which flows through the muskeg produces local diversity in the vegetation. On the low banks are found *Alnus crispa*, associated with dense tussocks of *Carex leptalea*, *C. paupercula*, and *Caltha palustris*.

Peat Bog

From the detailed examination of several peat bogs in the region two general observations about their structure emerge: that there are three intergrading zones of bog vegetation in addition to that region which might be described as the peat bog/muskeg ecotone; and that within these zones a mosaic of vegetation units can be recognized which, by their relative abundance, characterize the three main zones.

The three strictly peat bog zones will be described from a series of three bogs at Station 12 (Fig. 3); they are representative of the 13 stands of peat bog which were examined in the areas of study.

The first zone consists of an open community of colonizing species, found in the wettest, generally central parts of the bog; here are found the earliest stages of bog development characterized by the complete absence of a *Sphagnum* mat; the following were recorded:

Menyanthes trifoliata	– a	*Eriophorum angustifolium*	– f
Comarum palustre	– f	*E. medium*	– o
Carex rostrata	– o	*Drepanocladus aduncus*	– f (la)

*= *Arctostaphylos rubra.*
†= *Ranunculus lapponicus.*

The second zone is diverse but it might be described as the zone of closed vegetation characterized by a submerged, loose mat of hypnoid mosses associated with a sedge-dominated community. The outer margin of this zone, contiguous with the first zone, consists entirely of *Carex rostrata* growing in a loose mat of *Drepanocladus aduncus*, *D. intermedius*, and *Meesia triquetra*. Where this moss mat is more stable the following species are found:

Carex aquatilis	– o, lf	*Triglochin maritima*	– o
C. chordorrhiza	– o	*Smilacina trifolia*	– lf
C. diandra	– a	*Scheuchzeria palustris* var. *americana*	– r
C. limosa	– a	*Platanthera hyperborea**	– f
Eriophorum brachyantherum	– o	*Stellaria longifolia*	– o

Pioneer hummocks of *Sphagnum warnstorfianum* occur in this zone, together with *Salix pedicellaris* var. *hypoglauca* and *Betula glandulosa*.

The third zone consists of a hummocked mat of *Sphagnum* bearing a characteristic vegetation of *Andromeda polifolia*, *Oxycoccus microcarpus*, *Carex heleonastes*, *C. tenuifolia*, *Salix pedicellaris* var. *hypoglauca*, and *Rubus acaulis*. The species of *Sphagnum* which make up these hummocks are most commonly *S. warnstorfianum* and *S. squarrosum*, and they are replaced locally by *Camptothecium nitens*. The moss hummocks are often large—reaching 60 cm. in diameter and 15–25 cm. in height—and they are separated by low, wet interstices in which the plants enumerated for zones one and two are found, the exact floristic composition depending on the level of the water. The periphery of the *Sphagnum* mat (and of the whole bog) is formed by a narrow microphanerophytic zone consisting of *Betula glandulosa* and *Chamaedaphne calyculata* var. *angustifolia*.

In one of the peat bogs at Station 12 (Fig. 3) local conditions of substratum effect a change in the vegetation. At the east end of the bog, adjacent to the muskeg/spruce forest transition, there is a considerable mineral element in the substratum. Dominating this zone is *Calamagrostis canadensis*, with *Salix pyrifolia* and *S. arbusculoides*. This facies of the bog is immediately contiguous with the raised clay hummock/muskeg vegetation which has been described above. In several of the bogs which were studied *Larix laricina* was observed to occupy sites of varied ecological character. It occurs, usually singly, throughout the bog zones but appears to die out in well developed *Sphagnum* mats, being most vigorous in the wetter moss–sedge zone. However, the ecological relationships of larch in these regions are not immediately apparent and will be considered in detail below.

At Station 12 (Fig. 3) a bog occurs through which a small stream flows. The mineral soil associated with this water movement effects the appearance of certain species which are not completely typical of peat bogs: *Viola palustris*, *Epilobium palustre*, *Senecio pauperculus*, and *Cicuta mackenzieana*. However, these silting effects are local and the greater area of the bog consists of vegetation which accords with the description outlined above.

The above descriptions of muskeg and peat bog accord well with those of Raup (41), Lewis, Dowding, and Moss (28), and Moss (34) for northwest

*= *Habenaria hyperborea*.

Canada, and with those of Hustich (19, 20) for certain regions in Quebec. They differ from those of Dansereau (7, p. 210), Dansereau and Segadas-Vianna (8), and Segadas-Vianna (52) for southern regions of Quebec in that there *Chamaedaphne calyculata* var. *angustifolia* appears to form a more conspicuous part of the bog structure; in the present area and in northern and western bogs generally, it seldom forms large pure stands and its role in bog communities appears to be taken over in part by *Ledum groenlandicum* and *Betula glandulosa*.

Lake Vegetation

Aquatic

There is little macrovegetation in deep, steeply shelving lakes, such as Tod Lake; however the scant marginal and outcrop communities of the shore lines of these lakes will be considered below in this section. Lakes with shallow marginal zones have well developed aquatic communities; all of the communities which are described below are located on various parts of the MacBride Lake and MacBride River drainage system of Fig. 3.

Where the shore line consists of gently sloping drift material there is a continuous zonation from the open water community to the marginal forest fringe. In the shallowest bodies of water, which are being filled in by aquatic and semiaquatic vegetation, there is a well developed zone of submerged plants. The shallow southern arm of MacBride Lake illustrates this well and the following plants were noted there in $\frac{1}{2}$–1 m. of water:

Potamogeton robbinsii	– d	*Batrachium subrigidum*	– lf
P. alpinus var. *tenuifolius*	– f	*Drepanocladus sendtneri*	– a

These plants form a dense, submerged zone. In slightly shallower water, at about 50 cm., they are replaced by dense stands of *Nuphar variegatum*, associated in shallower water with *Hippuris vulgaris*, *Utricularia vulgaris*, and *Potamogeton richardsonii*. At this depth there is a thick mat of decayed organic matter on the lake bottom and in shallow, marginal areas *Menyanthes trifoliata*, *Calla palustris*, *Carex vesicaria*, and *Caltha palustris* become common. This zone abuts upon the drier, mineral soil of the lake shore.

In certain areas of this arm of MacBride Lake the process of filling-in of the lake proceeds more rapidly and a local zone of peripheral bog vegetation develops. This is most conspicuous at the mouth of a small, slow stream, along which there are several beaver dams; the silt and plant debris which is carried along the stream and accumulates in the lake accelerates the establishment of bog conditions. Behind the submerged and floating-leaf zones, there is a loose, partly consolidated mat of peaty debris; the outer fringe of this is occupied by *Eleocharis acicularis*, *Menyanthes trifoliata*, *Carex vesicaria*, *Lycopus uniflorus*, and *Caltha palustris*. Behind this zone is a broad zone dominated by *Carex lasiocarpa* var. *americana*, *Comarum palustre*, *Rumex* sp., *Equisetum fluviatile*, *Caltha palustris*, and *Cicuta mackenzieana;* locally *Calamagrostis canadensis* assumes dominance.

The small bay at Station 8 (Fig. 3), adjacent to one of the organic ridges dominated by *Betula papyrifera* var. *neoalaskana*, consists of irregularly

shaped mats of this lake-bog vegetation. By definition, it is a rather atypical bog, since there is no *Sphagnum* mat, and ericoid shrub zones are absent; however, in other areas there are true bog zones indicating that this lake-bog type is relatively immature and that the presence of more typical swamp plants and the absence of characteristic bog phases is determined by the presence of fluvial silt in the lake deposit. It would seem to be reasonable to conclude that the later stages of filling-in of lakes are represented by the invasion by *Sphagnum* with the development of more typical bog zonation. There are several areas in this region which indicate that this was the sequence of vegetation.

In deeper lake basins, with more abruptly inclined sides, there is less rapid colonization by vegetation and little peat accumulation. Here the outer aquatic community in shallow (1 m.) water is made up of *Nuphar variegatum*, *Potamogeton richardsonii*, *Sparganium angustifolium*, *Sagittaria cuneata*, and *Myriophyllum exalbescens*. In shallower water ($\frac{1}{2}$ m.) there is often a zone of *Equisetum fluviatile* and along the shallow margins there is a narrow zone of *Carex rostrata*, generally associated with *Naumbergia thyrsiflora** and *Sium suave*. *Callitriche palustre* was recorded from one shore line community in about 40 cm. of water.

The Vegetation of Lake Margins

On drift substrata, lakes are often fringed by discrete, though usually discontinuous communities. On low stony clay shores at the water's edge a shrub zone is common, of which *Salix bebbiana*, *Myrica gale*, *Alnus crispa*, and *Cornus stolonifera* are common (Fig. 8). Behind this shrub zone there is sometimes a transition zone of mixed forest between it and the forest type of the ridges; the extent of this zone depends on the soil drainage, and in areas where there is a low flat marginal region with impeded drainage the peripheral zone is 10–15 m. in width. It is dominated by well grown trees of *Salix bebbiana* which reach 10 m. in height and 15 cm. in trunk diameter and which often are associated with *Populus balsamifera*. The following list was compiled from this marginal copse type at Station 15 (Fig. 3):

Salix bebbiana	– a	*Equisetum fluviatile*	– lf
Betula papyrifera var. *neoalaskana*	– f	*Rosa acicularis*	– o
Populus balsamifera	– o	*Fragaria vesca*	– o
Larix laricina	– o	*Actaea rubra*	– o
Picea mariana	– o	*Chamaepericlymenum canadense*	– o
Alnus crispa	– f	*Mertensia paniculata*	– f
Cornus stolonifera	– o	*Aralia nudicaulis*	– f
Myrica gale	– f	*Viburnum edule*	– o

In other areas the lake margins slope more steeply and the forests extend to the edge of the lakes with little zonation. In such cases there is a characteristic group of plants which occupy the shaded, damp habitats on humous-covered shore line boulders. These are: *Parnassia palustris* var. *neogaea*, *Epilobium palustre*, *Lycopus uniflorus*, *Climacium dendroides*, *Mnium cinclidioides*, and *Dicranum strictum*. Very locally small swards of *Ranunculus*

* = *Lysimachia thyrsiflora*.

reptans and *Eleocharis acicularis* were recorded on wet clay between drift boulders just above the water level; here they are associated with *Carex rostrata*, *Naumbergia thyrsiflora*, and *Sium suave*.

Shore Line Outcrops

Communities on shore line outcrops in this area show some floristic variation but the ecological conditions appear to be more or less similar throughout. These habitats consist of exposed, open rock which is generally covered in part by a layer of humous soil. The adjacent forest vegetation seldom extends onto the small shore line outcrops, which remain open and little shaded. The precise floristic composition of the outcrops would appear to be influenced by the degree of insolation and the amount of soil which develops over them. For example, a particularly well sheltered, small (about 12 sq. m.) outcrop on an arm of MacBride Lake (Station 9, Fig. 3), which terminated a small shore line promontory and was therefore exposed to considerable insolation, showed a relatively rich flora on a shallow humous substratum; the following plants were recorded:

Juniperus communis var. *depressa*	– f	*Saxifraga tricuspidata*	– o
Alnus crispa	– one plant	*Chamaenerion angustifolium*	– r
Rubus pubescens	– one plant	*Linnaea borealis* ssp. *americana*	– o
Arctostaphylos uva-ursi var. *adenotricha*	– o	*Solidago multiradiata*	– r
Botrychium lunaria	– r	*Achillea millefolium*	– r
Woodsia ilvensis	– r	*Hieracium umbellatum*	– r
Trisetum spicatum var. *molle*	– r	*Camptothecium nitens*	– f
Poa glauca	– o	*Thuidium abietinum*	– o
		Cladonia rangiferina	– f

A small, steeply sloping outcrop (granite) on MacBride River (Station 15, Fig. 3), with a western aspect had a less varied flora; the following plants were recorded: *Juniperus communis* var. *depressa*, *Trisetum spicatum*, *Poa glauca*, *Carex media*, *Geocaulon lividum*, *Saxifraga tricuspidata*, and *Arnica lonchophylla* var. *lonchophylla*. In crevices of an adjacent, steeply sloping outcrop on this shore line *Polypodium virginianum* var. *americanum* and *Potentilla norvegica* were noted.

Minor Communities

Throughout the areas of study, within the broad vegetation types which have been described, various small assemblages of plants were noted, which were associated, apparently, with local habitat conditions. These will be described briefly in this section.

The general topographical nature of the area is such that there are few abrupt features of relief. However, one ridge, by reason of a local faulting shows an abrupt relief, consisting of a high summit with steep sides; this yields a number of atypical habitats. This is the ridge which bounds the northwest side of Tod Lake (Fig. 2, Station 2), and to which reference has been made. The flanks bear small cliff and crevice habitats in which certain rare (for this region) plants were found. The open cliff habitats on the south-facing side of the ridge yielded the following group of plants: *Woodsia ilvensis*, *Schizachne purpurascens*, *Roegneria violacea*, *Carex abdita*, *Arabis holboellii* var. *collinsii*, *Potentilla pensylvanica* var. *pensylvanica*, *Oxytropis splendens*, *Artemisia caudata*,

and *Solidago decumbens* var. *oreophila*. These plants were found in thin soil of crevices on the summits and sides of the small cliffs. In moderately shaded sites, on the south-facing side but with some shelter from direct sunlight, the following were recorded: *Cystopteris fragilis* and *Dryopteris fragrans*. In crevices of small outcrop cliffs on the north-facing, shaded slopes of this ridge, under a closed canopy of black spruce, the following were noted: *Polypodium virginianum* var. *americanum*, *Woodsia alpina*, *Cystopteris dickieana*, *C. fragilis*, *Saxifraga tricuspidata*, *Nephroma helvetica*, and *Parmelia stenophylla*. Many of these plants recorded on the cliffs of this ridge were not found elsewhere in the region; they are species of relatively narrow ecological amplitudes, usually confined to such habitats.

Adjacent to this ridge a small stream which had been incompletely dammed by beavers yielded a local society of plants. Beside the stream, in rich muck soil, were *Alnus rugosa* var. *americana*—the only record from the region—*Cornus stolonifera*, and *Populus balsamifera*, while in the rich, wet, shaded soil round the small dam *Thelypteris phegopteris*, *Glyceria striata* var. *stricta*, *Mitella nuda*, *Aralia nudicaulis*, and *Mertensia paniculata* were recorded.

Another minor, and exceptional community was studied at a point inland from the northwest shore of Tod Lake (Station 1, Fig. 2). This was represented by two small depressions, each approximately 15 × 25 m. on a clay till ridge dominated by the seral open pine forest type which has been described in detail above. The outer zone of the depression is occupied by individuals of *Salix pyrifolia* with a dense carpet of *Polytrichum formosum* on the ground. Within this is a concentric zone dominated entirely by *Juncus filiformis* and *Carex oligosperma* which grow together in approximately equal proportions; the center of the area is occupied by a hummocked mat of *Sphagnum capillaceum* with *Calamagrostis lapponica* var. *nearctica*. The precise interpretation of this local community is obscured by the fact that the area has been subject to forest fire, and it is clear from the impoverished condition of the vegetation and the absence of any other species that these depressions have undergone considerable desiccation. It seems probable that they are in a degeneration phase (after fire), and they might be assigned to that type of intermediate community which shows physiographic and vegetational features in common with both bog and swamp.

V. Discussion

In eastern Canada various terms are in use as alternatives to Halliday's (11) northern coniferous section of the boreal forest, in particular the southern spruce forest of Hustich (19), the main boreal forest of Hare (13), and the temperate coniferous forest of Rousseau (47). Nevertheless there is a considerable degree of agreement about the type of vegetation to which these terms refer. The present writer uses Hustich's (19) term. This zone is characterized by a forest on mesic sites which consists of a closed canopy of spruce with no other tree or tall shrub stratum. The southern spruce forest, which in Manitoba is identical with the B 21 and B 22 zones of Halliday (11) and the Northern Coniferous Belt of Harrison (16, map, p. 19), is replaced

in the north by the taiga (Hustich (19)), alternatively designated the northern transition zone (B 27) by Halliday (11), the open boreal woodland by Hare (13), or the subarctic parkland by Rousseau (47). The main physiognomic difference between the types is that the taiga is a forest with a discontinuous or open tree stratum while the southern spruce forest is a completely closed community. The precise boundary between these two zones has been defined by Hare and Taylor (15), who revise Hare's (14) earlier suggestion; these authors indicate that that vegetation of which no more than 10% of the total forest cover is of the "open boreal forest type" lies within the southern spruce forest zone. By this criterion, and any other, both the areas with which this study is concerned are within the southern spruce forest type, since at no point was any forest on a mesic site noted which could be assigned to the taiga type. It should be noted here that this conclusion indicates that the boundary between the taiga (B 27) and the southern spruce forest (B 22) which Halliday (11) has plotted is inaccurate in the region immediately east of Lynn Lake; according to the map (11) the MacBride Lake area would be included in the taiga. However, the error must be slight since Baldwin's (1) studies at Reindeer Lake, at a point about 100 km. northwest of MacBride Lake, reveal that ". . . an open parklike forest . . . is characteristic of the sites above the level of swamp and muskegs" (1, p. 115); that is, the area is within the taiga. This would suggest, as Hare and Taylor (15) have shown in Quebec–Labrador, that the transition between the southern spruce forest and the taiga is abrupt.

Within the southern spruce forest zone—and for the forests of the Quebec–Labrador peninsula in general—Hustich (19) makes three subdivisions of the vegetation on the basis of habitat, recognizing "dry", "moist", and "wet" series; these are homologous with xeric, mesic, and hydric and they provide a useful basis for reviewing the vegetation of the present area. With the limited and highly local information which is available from the present work it is not possible to extend further this classification of habitat, as Hills (17) and Rowe (48) have done for the mixed forest sections of the boreal forest. In order to secure some estimate of the relative areas occupied by the major community types found on these habitats, vertical aerial photographs were used to construct preliminary vegetation maps, and the areas of the types were measured on a grid. (This work is being carried into considerably greater detail and will be reported in a future account.) The figures provided below, percentages of the total area of vegetated terrain, refer to the region mapped in Fig. 3.

Zonal type	Habitat type	Vegetation type	%
Southern spruce forest zone	Xeric	Jack pine forest on eskers and outcrops	7
	Mesic	Closed black spruce forest and seral types	66
		White spruce forests	1
	Hydric	Muskeg	21
		Peat bog	4
		Lake vegetation	2

Clearly, for this region, the most advanced forest type of mesic habitats—that is, sites with moderately drained substrata and well developed soils—is the closed black spruce forest, although it is represented on many sites by subseral types. This mature forest is identical in every respect with the "conifer feather moss forest" of Hustich (19) and the "black spruce – feather moss association" of Moss (33). Of this forest Hustich (19) writes (p. 39): "If there are such things as climax forests in nature, the spruce feather moss forest is certainly one of them", and for this area at least the present author would accord with this view. Moss (33), whose studies are largely in the mixedwood section (B 18) of the boreal forest, describes white spruce forest as "the climax association of mesic sites", a condition which prevails in the B 18 of Manitoba (Halliday (11)); and only from the extreme northwest of Alberta does Moss (33) describe forests on mesic sites which are dominated by black spruce. At least for this region the southern spruce forest zone is characterized on upland mesic sites by a considerably greater proportion of forests dominated by *Picea mariana* than by *P. glauca*. The present study would indicate that the latter are rare and local, being confined to sites with optimum conditions of soil drainage and aspect. For comparable areas of the southern spruce forest zone in Quebec, Hustich (19) indicates that black spruce forest is considerably more common that white spruce types. And he points out that *P. mariana* is the typical dominant of the "conifer feather moss forest" type (19, p. 39). Of the areas of the southern spruce forest in Quebec, the Central Laurentian Section of Hustich (19) and Halliday (11), also the B 3 zone of the latter author, would appear to bear the closest resemblance to the present area. And the descriptions by Halliday (11) of the southern spruce forest in Ontario (B 4 and B 8), and those of Hutchinson (23), indicate that the general pattern of vegetation types is similar to what has been described here. Scoggan (51, p. 146) describes black spruce as the main tree of upland sites in the B 22 zone near Hayes River, East Manitoba, and he notes that "where soil conditions are more favourable, mixed stands of white-spruce, white birch, balsam fir, aspen and balsam poplar occur . . .". Pulling's (37) descriptions of the forest cover of areas of northern Manitoba at the same latitude as the region described here are identical to those outlined above. Tyrrell (55) describes an essentially similar vegetation pattern for the country between Churchill River and Athabasca Lake, of which he notes (p. 12D) that "White spruce forms some groves of a fair size in the bottom lands near the Churchill River, but further north it is rarely seen except in some particularly favourable localities". He points out that jack pine is most commonly found on sand plains and that the main tree of the area is black spruce. This is scarcely compatible with the account of the B 22 region between Athabasca Lake and northwest Saskatchewan by Raup (44) who indicates (map, p. 62) that the area is dominated by jack pine forest which, while in certain regions is a seral (fire) type, on the pre-Cambrian areas is natural forest and "for the time being at least is succeeding itself". Further west, on the Palaeozoic areas of the Interior Plains, white spruce forest is described as the dominant

of mesic sites (Raup (44) and Halliday (11)); this is the B 23 of the latter author. In the same region a "park-like white spruce" abuts on the northern boundary of the B 22 and B 23 zones (Raup (44, map, p. 62)); and the descriptions of this zone accord with those of other authors for the taiga of eastern Canada. Raup (43), Denny (9), and Raup and Denny (45) have shown that, in parts of northwest Canada there is a marked coincidence of black spruce feather moss forest with areas of heavy clay soils. (It seems probable that any further intensive work in the present area might profitably investigate the role of edaphic factors, in particular soil texture, moisture regime, and permafrost, in determining the patterns of vegetation.)

It would appear to be possible, therefore, to extend Hustich's (19) southern spruce forest zone from Quebec at least as far as the western boundary of Manitoba, and its area would coincide, for the most part, with the B 21 and B 22 zones of Halliday (11). The characteristics of the zone are the preponderance, on mesic sites, of a closed forest of *Picea mariana* with no subsidiary stratum other than a dense ground carpet of weft- and turf-form mosses, and the restriction of mixed, white spruce forests to sites which show optimum habitat conditions. It should be noted here that in southern parts of this southern spruce forest zone *Abies balsamifera* is often associated with both *Picea mariana* and *P. glauca* (more frequently the latter) in certain habitats; however, it is absent from the present area of study, its northern limit lying to the south of the northern limit of the southern spruce forest (Hustich (20)) and not determining the northern boundary of that zone as Connell (6, p. 5) would suggest.

While the closed black spruce forest is the most mature or the stable forest of mesic sites, it has been shown in the foregoing descriptive account that much of the area of these habitats is dominated by subseral forests. It has been suggested that the mosaic of varied forest types which is met with on mesic sites can be interpreted satisfactorily in terms of relative age after burning, the precise nature of the vegetation being determined by the incidence and severity of fire, the nature of the forest at the time of the fire, and the type of adjacent vegetation which will supply many of the recolonizing shrub and tree individuals. Extensive areas, particularly north of the MacBride River and MacBride Lake, are dominated by the open pinewood or jack pine parkland forest type. It would appear that this feature of the vegetation occurs in western parts of the B 22 to a considerable extent (cf. Halliday (11, p. 21) and Raup (44)), although this would appear to contradict the data of Halliday and Brown (12, map, p. 360) for jack pine intensity. It is suggested here that at least for areas where black spruce forest is clearly the most advanced type on mesic sites, the dry climate (cf. Sanderson (50)) which is prevalent in areas west of the present locale, and, as was noted above, for which there is some evidence here, retards the seral process by which black spruce forest replaces jack pine communities after fire. LeBlanc (27, p. 372), interprets the persistence of patches of jack pine forest in a regenerating (after fire) black spruce forest region as ". . . . showing a much more severe destruc-

tion of the humus layer". And it is well established that the optimum conditions for the formation of humous horizons in mineral soils are those of cool and humid conditions. This view that the increasing dryness of the climate might favour the persistence of subseral pine parklands would appear to corroborate the suggestions of Sanderson (50) concerning the effects of drought on the vegetation of the Canadian northwest; she indicates that the preponderance of open prairie and parklike communities might well be related to the drought factor.

In this connection, as has been noted elsewhere (Ritchie (46)), it might be relevant to refer briefly to the unusual group of plants which was recorded on the exceptional, high ridge at Station 2 (Fig. 2), to which reference has been made in the descriptive sections above. These plants—*Carex abdita, Arabis holboellii* var. *collinsii, Potentilla pensylvanica* var. *pensylvanica, P. arguta, Amelanchier alnifolia,* and *Oxytropis splendens*—are typical members of the deciduous forest and prairie zones in Manitoba, growing in open, well drained habitats. Their occurrence here is considerably north and east of their main areas of distribution. At present they are confined to that rare and atypical ridge habitat which shows optimum conditions of climate and soil, and it is suggested that the presence of this fragmentary southern and western element is symptomatic of the slight tendency towards dryness of climate.

It is apparent that the little known vegetation described above from the region north of MacBride Lake, consisting of a mosaic of pine forest on upper slopes and open, 'heathy' tracts on the middle and lower slopes, might be the source of profitable study. The present tentative interpretation is that it is primarily a subseral community; however, it appears that forest recolonization is slow and that the environment of certain areas is inimical to tree growth. These areas are occupied by an unusual heath/herb/lichen phase which bears slight physiognomic and floristic resemblances both to the ground vegetation of certain southern parkland spruce forests on dry sandy substrata, and to the heathy tundra of arctic regions. It is possible that local pockets of frost, or even of perennially frozen ground, restrict the establishment of trees. Here it should be noted that since the areas of study lie near the southern limit of permafrost (Thomas (53, p. 193)) the small number of observations of this phenomenon which were made provide an inadequate basis for evaluation of the significance of this factor.

From a consideration of the autecology of certain tree species the conclusion emerges that competition is often the factor which determines the structure of particular communities. For example as Hutchinson (23, p. 489) pointed out many years ago, *Pinus banksiana* would appear to grow in these habitats from which competition from *Picea mariana* is absent—that is on extremely xeric substrata and in certain seral communities. But its occasional occurrence in hydric sites (here in muskeg) is evidence that its range of tolerance of edaphic conditions is at least as great as that of black spruce. It would appear that a similar, though less conspicuous, situation obtains for *Larix laricina.* It was observed in the area over a wide range of soil types, from moderately

drained upland mineral soils (very rare) to the wettest peat substrata. Macoun (29) notes that, in the north, it occurs on dry sites, and it is possible that its absence from the majority of xeric and mesic sites is due to its inability to compete with other trees and shrubs rather than a restricted range of edaphic tolerance (cf. Beeftink (2)).

In this area *Populus tremuloides* shows a predilection for particularly mesic sites. It was present in greatest abundance in local areas which had been burned on the high ridge which flanks the northern shore of Tod Lake. In more southern regions it appears that it is an important subseral type, and it is of interest to note that Nash (36) observed, in the Cross Lake area about 350 km. southeast of this region, that it is a conspicuous dominant after fire "in the areas of best soil and shelter and greatest insolation while rocky areas of lower insolation favor the production of birch". Aspen, like white spruce, becomes progressively less common in northern areas of the coniferous zone where its place in the burn subsere is taken over largely by white birch. Almost without exception, in the present area of study, the pattern of occurrence of *P. tremuloides* is identical with that of *Picea glauca*.

Summary

(1) From detailed descriptions of the plant communities of two relatively small areas in northwest Manitoba it has been possible to establish the zonal vegetation type for the region. It would appear that the Southern Spruce Forest, which has been distinguished in eastern Canada, might be applied with justification to the present area.

(2) A forest with a single subsidiary stratum consisting of a dense carpet of weft-form mosses, and a closed canopy of *Picea mariana* is described as the predominant, stable type on mesic sites—in this region characterized by heavy clay soils. Owing to the high incidence of forest fires this type is represented in many areas by various seral types, which have been described.

(3) The occurrence of *Picea glauca* in the area is associated with local conditions of exceptional topography; the communities dominated by this tree are rare and appear to be confined to habitats of particularly favourable conditions of soil and climate.

(4) A description is provided of an unusual, and apparently undescribed community which was found on very local ridges of organic material. These sites are dominated by stands of *Betula papyrifera* var. *neoalaskana* which appear to be stable.

(5) Xeric habitats—outcrops, sand plains, and eskers—bear forest types which are dominated by *Pinus banksiana*. Muskeg, peat bog, and lake communities are described from various hydric sites.

(6) It is suggested that the presence of certain exceptional elements of vegetation and flora might be explained in terms of both locally unusual conditions of substratum and a slight tendency towards dryness of climate, this latter characteristic becoming more conspicuous in regions to the west of the present area of investigation.

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References

1. BALDWIN, W. K. W. Botanical investigations in the Reindeer–Neultin Lakes area, Manitoba. Natl. Museum Can. Bull. 128 : 110-142. 1953.
2. BEEFTINK, H. H. Some observations on tamarack or eastern larch (*Larix laricina* Koch.). Forestry Chron. 27 : 38-39. 1951.
3. BRAUN-BLANQUET, J. Plant sociology. McGraw-Hill Book Company, Inc., New York. 1932.
4. CAIN, S. A. The climax and its complexities. Am. Midland Naturalist, 21 : 146-181. 1939.
5. CAIN, S. A. Characteristics of natural areas and factors in their development. Ecol. Monographs, 17 : 185-200. 1947.
6. CONNELL, A. B. The forest types of Eastern Saskatchewan: an analytical classification. F. E. Thesis, University of Toronto, Toronto, Ontario. 1921.
7. DANSEREAU, P. Description and recording of vegetation upon a structural basis. Ecology, 32 : 172-229. 1951.
8. DANSEREAU, P. and SEGADAS-VIANNA, F. Ecological study of the peat bogs of eastern North America. I. Structure and evolution of vegetation. Can. J. Botany, 30 : 490-520. 1952.
9. DENNY, C. S. Late Quaternary geology and frost phenomena along Alaska Highway, Northern British Columbia and Southeast Yukon. Bull. Geol. Soc. Amer. 63 : 883-922. 1952.
10. GIMINGHAM, C. H. and ROBERTSON, E. T. Preliminary investigations on the structure of bryophytic communities. Trans. Brit. Bryol. Soc. 1 (4) : 330-344. 1950.
11. HALLIDAY, W. E. D. A forest classification for Canada. Dept. Mines and Resources, Canada, Forestry Service Bull. 89 : 1-50. 1937.
12. HALLIDAY, W. E. D. and BROWN, A. W. A. The distribution of some important forest trees in Canada. Ecology, 24 : 353-373. 1943.
13. HARE, F. K. Climate and zonal divisions of the Boreal Forest formations in eastern Canada. Geograph Rev. 40 (4) : 615-635. 1950.
14. HARE, F. K. The boreal conifer zone. Geograph. Studies, 1 : 4-18. 1954.
15. HARE, F. K. and TAYLOR, R. G. The position of certain forest boundaries in southern Labrador–Ungava. Geograph. Bull. In press.
16. HARRISON, J. D. B. The forests of Manitoba. Dept. Interior Canada, Forestry Service Bull. 85 : 1-80. 1934.
17. HILLS, G. A. A classification and evaluation of site in forestry. Ontario Dept. Lands and Forests, Div. Research, Research Rept. 24. 1952.
18. HUSTICH, I. Phytogeographical regions of Labrador. Arctic, 2 : 36-42. 1949.
19. HUSTICH, I. On the forest geography of the Labrador Peninsula. Acta geograph. 10 (2) : 1-63. 1949.
20. HUSTICH, I. The boreal limits of conifers. Arctic, 6 : 149-162. 1953.
21. HUSTICH, I. On forests and tree growth in the Knob Lake area, Quebec–Labrador Peninsula. Acta geograph. 13 (1) : 3-60. 1954.
22. HUSTICH, I. Forest-botanical notes from the Moose River area, Ontario, Canada. Acta geograph. 13 (2) : 3-50. 1955.

23. HUTCHINSON, A. M. Limiting factors in relation to specific ranges of tolerance of forest trees. Botan. Gaz. 66 : 465-493. 1918.
24. KILBURN, L. C. Geology of the MacBride Lake area, Uhlman Lake Division, Northern Manitoba. Manitoba Dept. Mines Nat. Resources, Mines Branch Publ. In press.
25. KIMBLE, H. T. and GOOD, D. *Editors*. Geography of the Northlands. Am. Geograph. Soc. Spec. Publ. No. 32. 1955.
26. KÜCHLER, A. W. Localizing vegetation terms. Ann. Assoc. Am. Geograph. 37 (4) : 197-208. 1947.
27. LEBLANC, H. A new approach to the Northern Spruce regeneration problem. Forestry Chron. 30 : 372-379. 1954.
28. LEWIS, F. J., DOWDING, E. S., and MOSS, E. H. The vegetation of Alberta. II. The swamp, moor and bog-forest vegetation of Central Alberta. J. Ecol. 16 : 19-70. 1928.
29. MACOUN, J. The forests of Canada and their distribution, with some notes on the more interesting species. Trans. Roy. Soc. Can. (4) : 3-20. 1894.
30. MARR, J. W. Ecology of the forest-tundra ecotone on the east coast of Hudson Bay. Ecol. Monographs, 18 (1) : 117-144. 1948.
31. MILLIGAN, G. C. Geology of the Laurie Lake area, Granville Lake Division, Northern Manitoba. Manitoba Dept. Mines Nat. Resources, Mines Branch Publ. 50-7 : 1-30. 1952.
32. MOSS, E. H. The vegetation of Alberta. J. Ecol. 20 : 380-415. 1932.
33. MOSS, E. H. Forest communities in northwestern Alberta. Can. J. Botany, 31 : 212-252. 1953.
34. MOSS, E. H. Marsh and bog vegetation in northwestern Alberta. Can. J. Botany, 31 : 448-470. 1953.
35. MOSS, E. H. The vegetation of Alberta. Botan. Rev. 21 (9) : 493-567. 1955.
36. NASH, J. B. An investigation of some problems of ecology of the Beaver in Northern Manitoba. M.Sc. Thesis, University of Manitoba, Winnipeg, Manitoba. 1951.
37. PULLING, M. E. Root habit and plant distribution in the far North. Plant World, 21 : 223-233. 1918.
38. RAUP, H. M. The distribution and affinities of the vegetation of the Athabasca – Great Slave Lake region. Rhodora, 32 : 187-208. 1930.
39. RAUP, H. M. The formation of peat ridges on the shores of muskeg lakes in northern Alberta. Rhodora, 33 : 18-23. 1931.
40. RAUP, H. M. Notes on the distribution of white spruce and Banksian pine in northwestern Canada. J. Arnold Arboretum (Harvard Univ.), 14 : 335-344. 1933.
41. RAUP, H. M. Phytogeographic studies in the Peace and upper Liard River regions, Canada. Arnold Arboretum Contrib. 6 : 1-230. 1934.
42. RAUP, H. M. Botanical investigations in Wood Buffalo Park. Natl. Museum Can. Bull. 74 : 1-174. 1935.
43. RAUP, H. M. Forests and gardens along the Alaska Highway. Geograph. Rev. 35 : 22-48. 1945.
44. RAUP H. M. Phytogeographic studies in the Athabasca – Great Slave region. II. J. Arnold Arboretum (Harvard Univ.), 27 : 1-85. 1946.
45. RAUP, H. M. and DENNY, C. S. Photointerpretation of the terrain along the southern part of the Alaska Highway. Geol. Survey Bull. 963*D* : 95-133. 1950.
46. RITCHIE, J. C. Additions and extensions to the flora of Manitoba. Rhodora. In press.
47. ROUSSEAU, J. Les zones biologiques de la Peninsule Quebec – Labrador et l'Hemiarctique. Can. J. Botany, 30 : 436-474. 1952.
48. ROWE, J. S. Uses of undergrowth plant species in forestry. Ecology. In press.
49. SANDERSON, M. The climates of Canada according to the new Thornthwaite classification. Sci. Agr. 28 : 501-517. 1948.
50. SANDERSON, M. Drought in the Canadian Northwest. Geograph. Rev. 38 : 289-299. 1948.
51. SCOGGAN, H. J. Botanical investigations along the Hayes River route, northern Manitoba. Natl. Museum Can. Bull. 123 : 139-161. 1951.
52. SEGADAS-VIANNA, F. Ecological study of the peat bogs of North America II. Can. J. Botany, 33 : 647-684. 1955.
53. THOMAS, M. K. Climatological Atlas of Canada. National Research Council of Canada, Ottawa. Bull. 3151 : 1-253. 1953.
54. THORNTHWAITE, C. W. An approach towards a rational classification of climate. Geograph. Rev. 38 : 55-94. 1948.
55. TYRRELL, J. B. Report on the country between Athabasca Lake and Churchill. Geol. Survey Can. Ann. Rept. 1-120*D*. 1895.
56. TYRRELL, J. B. Ice on Canadian lakes. Trans. Can. Mining Inst. 9 : 13-21. 1910.

THE DEVELOPMENT OF THE ASCOCARP IN PHAEOTRICHUM HYSTRICINUM[1]

By Margaret E. Barr[2]

Abstract

The ascocarp of *Phaeotrichum hystricinum* Cain and Barr is initiated by division of a cell of the vegetative hyphae to form a pseudoparenchymatous mass. The ascostroma differentiates into a dark, single-layered wall and a hyaline locule area. The ascogenous cells arise in the center of the locule, increase in size and number, and give rise to the asci. The asci are scattered in irregular fascicles in the locule and are thin-walled and deliquescent at maturity. The ascostroma lacks a pore. This is designated as the Phaeotrichum Developmental Type. Representatives of allied genera show a similar type of development.

Introduction

Phaeotrichum hystricinum Cain and Barr (3) was collected on porcupine dung (M.E.B. No. 1277) in Michigan in 1953. The ascospores germinated readily to produce a dark brown, slow-growing mycelium on agar. From hyphae in the agar a blackish-brown pigment dissolved, giving the colonies and surrounding agar a black tinge. Both submerged and aerial hyphae were much branched and coiled. A few abortive ascocarps, bearing long, dark appendages, were first formed in culture, but were sterile. Finally in a sector of a colony derived from a single spore there appeared numerous fertile ascocarps. It is suggested that this sector resulted from a mutation. In subcultures from the sector fertile ascocarps reached maturity within 40 days. The developmental sequence of the fungus could be established from sections of fertile ascocarps taken from the colonies.

Observations

The beginning of ascocarp formation occurs as the enlargement of a cell of a hypha, or of two or more cells after hyphal anastomoses have occurred. This almost invariably takes place within the agar, rather than in the surface growth. The enlargement and subsequent division of one or two cells is depicted in Figs. 1 and 2. The cells of the vegetative hyphae and enlarged cells are uninucleate. Shortly after the cell enlarges, however, rapid nuclear division occurs. The enlarged cell may reach a size of 10–12 μ in diameter and contain many nuclei before cell wall formation takes place. Walls delimit a portion of cytoplasm surrounding each nucleus, until a complex knot of cells is formed. Generally, when two cells enlarge together, only one of them continues to develop, as in Fig. 2. The other may divide once or twice and then cease further activity. However, both may continue to grow and divide, resulting in a pair of ascostromata lying together, each with its ascigerous locule.

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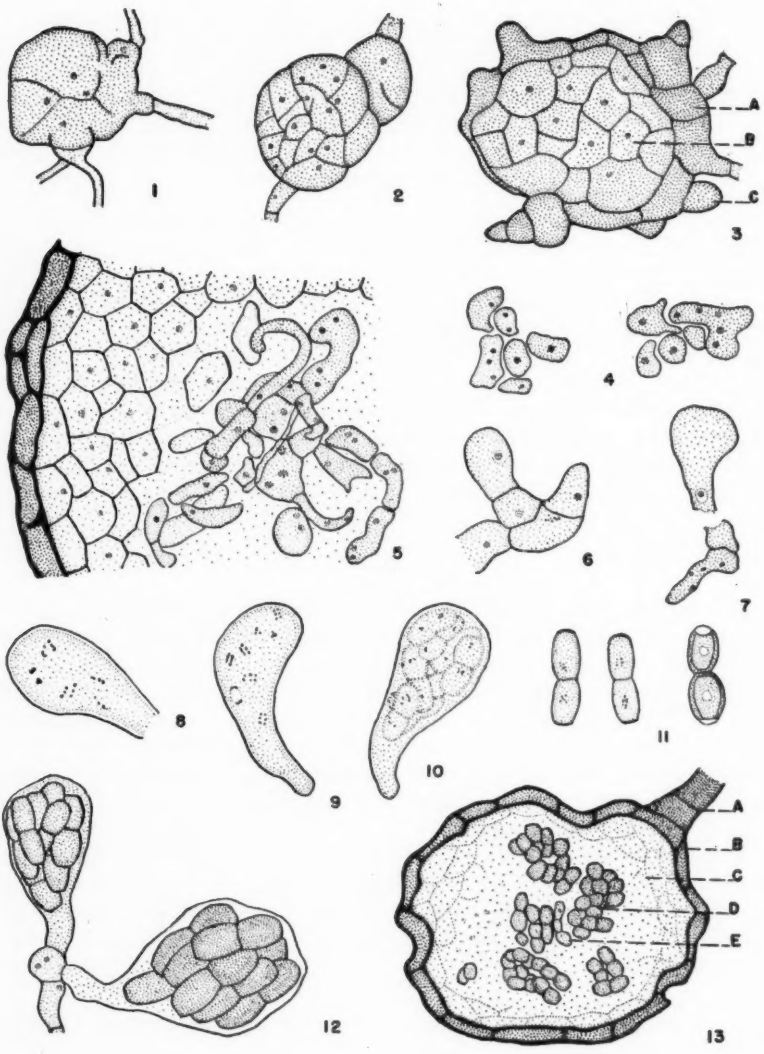
After a time, the knot of cells forms a stromatic mass. In sections there is visible externally a single layer of brown and thick-walled cells which form the wall of the ascostroma (Fig. 3A). Projections arise from certain of these cells and elongate to become the scattered appendages (Fig. 3C). In agar the appendages are flexible and hyphal, differing from the more rigid, tapering



appendages found on ascostromata in their natural habitat. Within the wall layer the cells (Fig. 3B) are thin-walled, irregular in shape, and contain a single nucleus in the granular cytoplasm. Most of the fertile ascostromata do not become over 70–100 μ in diameter. Those which become larger in general do not produce asci but remain filled with light-staining cells. Frequently sterile ascostromata are split almost into two by a wedge-like growth of dark wall cells into the locule.

As the fertile stroma continues to enlarge it retains the single layer of wall cells. Irregularly shaped, deep-staining cells now appear scattered in the locule (Fig. 4), and contain one to several nuclei. There does not appear to be any special arrangement of the ascogenous cells; their number increases from one or two to twelve or more. The deep-staining cells range in size from 3–5 μ, the size of the locule cells, up to 8–11 μ in length. As they increase in number, they may form a compact group in the center of the locule or they may extend from the center toward the wall layer (Fig. 5). Some of the cells become attenuated and twisted or coiled in the locule. In that case it is almost impossible to trace either their point of origin or their extremity.

Asci arise in small fascicles from the multinucleate ascogenous cells (Fig. 7). In some cases crozier-like structures are formed (Fig. 6) and the asci arise as outgrowths from the curved portion. In other cases, however, the asci appear to be cut off from the tip of an ascogenous cell or hypha. However they may arise, the asci are oblong, ellipsoid, or ovate, measure 8–11 μ in length by 3–4 μ in width, and contain one large fusion nucleus. A group of three to six will form a loose fascicle in which the apex of each ascus may point in a different direction. Each ascogenous cell may give rise to such a fascicle of asci. As the asci enlarge, they develop a narrow stalk and become broadly ovate to clavate. The fusion nucleus is granular in appearance and reaches a diameter of 1.5–2 μ, an increase over its size of 0.75 μ at the time it is first visible. The cells of the locule gradually disintegrate or are pushed aside by the developing asci.

Early stages of ascus formation, nuclear fusion, and meiotic division were extremely difficult to distinguish in sectioned material, so that squash mounts,

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FIGS. 1–13. *Phaeotrichum hystricinum.*

FIG. 1. Enlarged single cell of vegetative hypha. FIG. 2. Nuclear and cell divisions in one of two originally enlarged cells. FIG. 3. Section through a young ascostroma, showing the single wall layer, A; pseudoparenchyma of locule, B; appendage initial, C. FIG. 4. Two consecutive sections through ascogenous cells from the locule. FIG. 5. Section through a portion of locule and wall layer in relation to developing ascogenous cells and hyphae. FIG. 6. Ascus with fusion nucleus (upper left) developing from crozier (center) which has produced a second ascus (right). FIG. 7. Young ascus with fusion nucleus arising from ascogenous cell. FIG. 8. Four-nucleate ascus with the nuclei dividing mitotically. FIG. 9. Eight-nucleate ascus with the nuclei dividing mitotically. FIG. 10. Ascus containing eight binucleate spore initials. FIG. 11. Young and mature spores. FIG. 12. Two asci developed from a single ascogenous cell, with developing (left) and mature (right) spores. FIG. 13. Section through a mature ascostroma: A, base of appendage; B, wall of one layer of dark brown cells; C, disintegrating locule cells; D, grouped mature spores after deliquescence of ascus wall; E, young asci with fusion nuclei. (FIGS. 1–12, × 1425; FIG. 13, × 560).

stained in proprionocarmine, were utilized to obtain additional information. Separated ascogenous cells, containing several nuclei and producing asci, were found. Occasional elongate fusion nuclei, which suggest an early stage of first meiotic division, have been seen. A four-nucleate ascus is shown in Fig. 8. Here the minute chromosomes appear as granules grouped in various stages of division in the nuclei. The eight-nucleate stage (Fig. 9) likewise shows the nuclei during division. The chromosome complement appears to be $n = 3$. At the eight-nucleate stage faint walls may be distinguished delimiting oval portions of cytoplasm around each dividing nucleus. A later stage in development shows an ascus (Fig. 10) with eight spore initials, each of which is binucleate, and in some cases, has the central septum formed.

As the ascus develops the wall appears as a thin, non-staining region around the contents and narrows toward the base as the stalk. At maturity very little cytoplasm remains in the ascus surrounding the spores. Shortly after the spores are delimited they may be observed to contain a single nucleus in each cell. The nucleus is frequently granular in appearance (Fig. 11) and appears to be dividing. Later the nuclear material becomes compressed into a small mass. The wall becomes brown and thick as the spores mature, concealing the contents. The two ends of the spore are somewhat thickened and refractive when young. At maturity, the germ pores at each end of the spore appear as hyaline refractive hemispheres (Fig. 11). The ascus wall is very thin and can be seen only as a delicate refractive line around the maturing spores (Fig. 12). It is readily ruptured and the spores are freed within the cavity of the ascostroma (Fig. 13), from which they are finally liberated by breaking of the wall layer.

Discussion

The development of *Phaeotrichum hystricinum* is ascostromatic, and in its early stages is very similar to that in many members of the Loculoascomycetes. The asci, however, are borne in irregular fascicles scattered through the locule, and are thin-walled and deliquescent at maturity, features not found in the Loculoascomycetes. Several recent accounts of fungi considered as belonging to the Eurotiales indicate that there exist situations similar to that found in *Phaeotrichum hystricinum* within this order.

Stolk (7) has recently described *Westerdykella ornata*, the type of a new genus, isolated from East African mangrove mud. The ascocarps originate by the swelling of an intercalary cell of a hypha. The swollen cell divides several times to form a solid spherical body. In section, young ascocarps are composed of a hyaline pseudoparenchyma surrounded by a single layer of thick-walled brown peridial cells. No pore is formed. The ascogenous hyphae develop in the center of the pseudoparenchyma. Side branches from the ascogenous hyphae produce the stalked, subglobose to elliptical, deliquescent asci. The ascospores in this fungus are distinct from those of *Phaeotrichum* in being one-celled, light brown, and longitudinally striate; the ascocarps lack appendages.

The genus *Pseudeurotium*, broadened in concept by Stolk (6), now contains three species. The type species, *P. zonatum* van Beyma (2), and *P. ovalis* Stolk (6) are similar in development. The ascocarp initials arise from coiled hyphae and development is ascohymenial. Both species have a conidial *Sporotrichum* stage. The third species, *P. multisporum* (Saito and Minoura) Stolk (6) differs from the other two in the following respects: the ascocarp originates from an intercalary segment of the mycelium which swells and divides to form a solid pseudoparenchymatous mass; a conidial stage is lacking. *P. multisporum* is in development very similar to both *Phaeotrichum hystricinum* and *Westerdykella ornata*. The one-celled, smooth-walled ascospores distinguish *Pseudeurotium* from the other two genera.

The ascocarps of certain perfect stages of *Penicillium* in the course of development show similarities to the species previously discussed. The ascocarps of the forms segregated by Benjamin (1) into *Carpenteles* appear to be ascostromatic. They originate from the innermost branches in a network of hyphae. The branches anastomose, branch repeatedly, and intertwine to form a compact mass of pseudoparenchymatous cells. Nothing is definitely known of the earliest stages in initiation of the ascocarps, but sections of young ascocarps show a pseudoparenchymatic tissue of cells surrounded by a darker wall layer, and ascogenous cells in the center of the pseudoparenchyma. In the genus *Talaromyces*, erected by Benjamin (1) for a number of perfect stages of *Penicillium* which differ from those placed in *Carpenteles*, the ascocarps are initiated by a more or less well-developed ascogonium. The wall is composed of rather loosely interwoven hyphae surrounding the ascogonium. This type of development is ascohymenial and is similar to the condition in *Eurotium*.

The type of development described for *Phaeotrichum hystricinum* differs in several respects from the various developmental types described by Luttrell (4, 5) for the Ascomycetes. It is proposed that this type be designated as the Phaeotrichum Developmental Type, whose distinguishing characteristics are: the formation of ascostromata which remain completely closed, the development of thin-walled, unitunicate asci in fascicles scattered throughout the locule, and the deliquescence of asci at maturity. This type of development is known at present in members of the genera *Phaeotrichum*, *Pseudeurotium*, and *Westerdykella*, and may possibly occur in *Carpenteles*. The fungi which illustrate the Phaeotrichum Developmental Type are retained for the present in the Eurotiales. It must be recognized, however, that two distinct developmental tendencies occur within the order — ascohymenial and ascostromatic. In both types the asci are thin-walled. Detailed studies of representatives of all genera will be necessary before an adequate idea of the developmental and ascal types in this order are obtained, and before the true picture of their taxonomic affinities can be accurately expressed.

Acknowledgments

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References

1. BENJAMIN, C. R. Ascocarps of *Aspergillus* and *Penicillium*. Mycologia, 47 : 669-687. 1955.
2. VAN BEYMA THOE KINGMA, F. H. Beschreibung einiger neuer Pilzarten aus dem "Centraalbureau voor Schimmel-cultures" Baarn (Holland). IV. Mitteilung. Zentr. Bakteriol. Parasitenk. Abt. II, 96 : 411-432. 1937.
3. CAIN, R. F. Studies of coprophilous Ascomycetes II. *Phaeotrichum*, a new cleistocarpous genus in a new family and its relationships. Can. J. Botany, 34 (4). 1956.
4. LUTTRELL, E. S. Taxonomy of the Pyrenomycetes. Univ. Missouri Studies, 24 (3) : 1-120. 1951.
5. LUTTRELL, E. S. The ascostromatic Ascomycetes. Mycologia, 47 : 511-532. 1955.
6. STOLK, A. C. The genera *Anixiopsis* Hansen and *Pseudeurotium* van Beyma. Antonie van Leeuwenhoek J. Microbiol. Serol. 21 : 65-79. 1955.
7. STOLK, A. C. *Emericellopsis minima* sp. nov. and *Westerdykella ornata* gen. nov., sp. nov. Brit. Mycol. Soc. Trans. 38 : 419-424. 1955.

METABOLISM OF UNIFORMLY LABELLED MALTOSE-C^{14} INTRODUCED INTO DETACHED WHEAT AND TOBACCO LEAVES[1]

By G. Krotkov and S. Rizvi

Abstract

Uniformly labelled C^{14}-starch was incubated in the presence of saliva yielding radioactive maltose, which was isolated by paper chromatography. When this maltose was introduced into detached tobacco and wheat leaves, it was found to be respired and transformed into other carbohydrates. There was no evidence to indicate that maltose is either a better starch former than glucose or that it acts as a specific glucose donor to fructose in the synthesis of sucrose. No dilution in the specific activity of maltose was observed even under the conditions of starch hydrolysis. This indicates that hydrolysis of starch *in vivo* does not result in the addition of maltose to the metabolic pool of cells.

Starch is the commonest reserve carbohydrate in plants and under the action of amylases *in vitro* it is broken down to maltose. Since amylases are widely distributed in various plant organs, one may assume that they are also responsible for the breakdown of starch *in vivo*. If so, maltose should be a very common sugar.

Contrary to this expectation there are only occasional references in the literature to the occurrence of free maltose in higher plants. Maltose was reported in nasturtium leaves after a period of photosynthesis (2) and in a few other species of plants, especially when their detached leaves were partially dried to induce hydrolysis of starch (1).

The rare occurrence of maltose can be explained as being a result of its great activity brought about possibly by its glucosidic bond of about the same energy content as that of glucose-1-phosphate. Having such a bond, maltose may also act as a specific donor of glucose to fructose in sucrose synthesis.

The metabolic activity of maltose can be studied *in vivo* by the use of radioactive maltose, and the experiments reported below were carried out with this object in mind.

Materials

Mature tobacco leaves and two-week-old wheat seedlings were used as experimental material.* Individual plants of *Nicotiana tabacum* var. Connecticut seed leaf were grown in pots with garden soil under 16 hr. day in a constant light and temperature room. Variety Foundation Thatcher wheat was grown on garden soil in flats in the university greenhouse in the spring of 1955.

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Preparation of C^{14}-labelled Maltose

Starch uniformly labelled with C^{14} was extracted from tobacco leaves which were permitted to carry on photosynthesis in $C^{14}O_2$.† This was dissolved in water and digested with saliva at 37° C. for 24 hr. as described by Hassid *et al.* (5). The digest was spotted on Whatman 3 MM paper and run for 24 hr. in butanol : ethanol : water in the ratio of 52 : 32 : 16. A radioautograph obtained from such a chromatogram is shown in Fig. 1. The spot containing maltose was eluted and found to contain about 50% of the total activity present in the original starch.

FIG. 1. A radioautograph showing separated products of starch digestion by ptyalin.

†*Starch was kindly supplied by Dr. P. V. Vittorio.*

Experiment 1

This was conducted to compare the utilization of glucose and maltose as respiratory substrates. Four samples of two-week-old wheat seedlings, each weighing about 10 gm., were placed on 0.1 *M* solutions of either maltose or glucose* for 1 hr. in light and in front of an electric fan. At the end of this time the basal parts of the seedlings were washed with distilled water and the washings were added to the remaining solution to determine the amount of activity not taken by the seedlings. By subtracting the activity left in the solution from that offered to plants initially, one could calculate the amounts and activity in the sugars absorbed.

At the end of this one hour of sugar feeding, the plants were transferred to distilled water and kept for six hours in darkness in a stream of CO_2-free air. The CO_2 respired was absorbed in NaOH and its total amounts were determined by titration. CO_2 respired was precipitated as $BaCO_3$ on addition of $BaCl_2$, plated, and counted. The results of this experiment are given in Table I. Apparently both glucose and maltose of the same molarity served equally well as respiratory substrates.

Experiment 2

In order to observe the conversion of maltose to other carbohydrates, two tobacco leaves were put on 0.1 *M* maltose solution. Leaf No. 1 was left in air in light for one hour and leaf No. 2 for four hours. At the end of these periods each leaf was extracted with 80% ethanol. Alcoholic extracts were evaporated *in vacuo* to dryness, a known amount of water was added to each, and aliquots of the solution so obtained were spotted on 3 MM paper and chromatogrammed. Individual sugars were located by radioautography, eluted, and their activity and total amounts were determined. Starch was extracted from the alcohol insoluble residues of the leaves after solubilization with perchloric acid, and made to volume. Aliquots were plated and counted, and the total activity of starch was determined. A detailed technique for this analysis has been described elsewhere (7).

As is seen in Table II there was considerable conversion of maltose into other carbohydrates. Its conversion into sucrose, however, was 10 to 12 times greater than into starch. This is similar to what has been observed when C^{14}-labelled glucose or glucose-1-phosphate was fed to tobacco leaves (7). Free maltose, therefore, is no better starch former than glucose. The specific activities of the glucose and fructose moieties of sucrose were the same. In other words there is no evidence that, during sucrose synthesis, maltose acted as a specific donor of glucose to fructose in a manner similar to glucose-1-phosphate in *Pseudomonas saccharophila* (3).

**Uniformly labelled C^{14} glucose was kindly provided by Dr. P. V. Vittorio.*

TABLE I

UTILIZATION OF MALTOSE AND GLUCOSE AS RESPIRATORY SUBSTRATES

Sample (1)	Solution offered (2)	Specific activity of sugar, μc./mgm. of C (3)	Total CO_2 evolved, mgm. (4)	Total activity in CO_2, μc. (5)	Total CO_2 evolved as mgm. of C (6)	$C^{14}O_2$ from glucose-C^{14} as mgm. of C (7)	"Endogenous" respiration as mgm. of C (6–7) (8)
1	Water	—	14.95	—	4.08	—	4.08
2	Glucose (0.1 *M*)	0.580	23.74	0.74	6.48	1.28	5.20
3	Glucose (0.2 *M*)	0.308	34.29	0.66	9.36	2.14	7.22
4	Maltose (0.1 *M*)	0.445	23.74	0.60	6.48	1.35	5.13

TABLE II

TRANSFORMATION OF MALTOSE INTO OTHER CARBOHYDRATES BY TOBACCO LEAVES

Leaf No.	Total activity taken in, μc.	Activity in 80% EtOH extract, μc.	Activity in various carbohydrates isolated									T.A. transformed into other carbohydrates, μc.	Recovery of activity in isolated carbohydrates as % of T.A. taken in by the leaf
			Glucose				Fructose				Starch		
			Free		In sucrose		Free		In sucrose				
			T.A.*	S.A.*	T.A.	S.A.	T.A.	S.A.	T.A.	S.A.	T.A.		
1 (on maltose 1 hr.)	4.12	3.60	0.20	0.14	0.18	0.24	0.04	0.025	0.18	0.24	0.03	0.63	15.3
2 (on maltose 4 hr.)	13.13	9.52	1.13	0.29	0.45	0.45	0.99	0.27	0.45	0.45	0.07	3.09	23.5

*T.A. = *Total activity in μc.*
*S.A. = *Specific activity in μc./mgm. of sugar.*

TABLE III

EFFECT OF LIGHT AND DARKNESS ON THE DISTRIBUTION OF RADIOACTIVITY IN VARIOUS CARBOHYDRATES EXTRACTED FROM WHEAT LEAVES FED EITHER MALTOSE OR GLUCOSE

Sample No.	Total activity taken up, μc.	Treatment after feeding	Total activity in 50% EtOH extract, μc.	Per cent of total activity in 50% ethanol extract in the form of various sugars			
				Glucose, %	Fructose, %	Sucrose, %	Maltose, %
1 (0.1 *M* glucose)	4.77	Killed immediately after absorption	4.18	29.3	4.4	38.4	—
2 (0.1 *M* glucose)	3.74	Darkness 6 hr.	2.09	7.0	5.5	40.2	—
3 (0.1 *M* glucose)	4.63	Light 6 hr.	3.00	11.3	6.3	53.6	—
4 (0.1 *M* maltose)	4.05	Killed immediately after absorption	3.30	2.0	1.0	15.2	58.8
5 (0.1 *M* maltose)	3.45	Darkness 6 hr.	1.60	10.2	10.1	33.1	6.2
6 (0.1 *M* maltose)	4.65	Light 6 hr.	2.71	5.8	4.5	42.0	20.8

Experiment 3

In order to compare further the metabolism of maltose with that of glucose, six 5 gm. samples of wheat seedlings were placed in light for one hour on one or other of these two sugars. At the end of one hour some samples were extracted at once. Others were transferred to water and kept there for an additional six hours either in light or in darkness, and then were also extracted. All the extracts obtained were analyzed as described earlier with the results given in Table III.

One can see from Table III that sucrose was the main sugar formed both from glucose and maltose. For every pair of comparable leaf samples more sucrose was formed from glucose than from maltose. Light stimulated sucrose synthesis from either glucose or maltose.

Experiment 4

To observe if the breakdown of starch *in vivo* results in the production of maltose, two tobacco leaves were placed in light for one hour on a 0.1 *M* maltose solution with a specific activity of 0.216 μc./mgm. of sugar. At the end of this time one half of each leaf was cut off the midrib and extracted with 80% ethanol. The other half of the leaf, still attached to the midrib, was placed in air on water for six hours either in light or in darkness. Then each lamina was cut off the midrib and extracted with ethanol. All alcoholic extracts were chromatogrammed as described earlier, and the specific activity of the isolated maltose, as well as the total activity in starch, was determined.

As is seen in Table IV the specific activity of maltose isolated after the experiment was about the same as that of the maltose fed. During the experiment itself there was disappearance of starch in leaf 1 and considerable synthesis in leaf 2. The amounts of maltose incorporated into starch were small in both leaves. Similar absence of maltose dilution was also observed when radioactive maltose was fed to wheat leaves.

In view of the absence of dilution in the specific activity of the maltose fed one can postulate two courses for the breakdown of starch in such leaves. According to the one the breakdown of starch in leaves does not go as far as maltose. It stops somewhere earlier, since some maltose precursors, like low molecular weight dextrins or oligosaccharides, are so rapidly metabolized that normally they have no chance to produce maltose. Gibbs (4) did observe that in the early stages of photosynthesis glucose derived from dextrins became uniformly labelled sooner than free glucose. This worker suggested, therefore, that short chain dextrins appear to be very active metabolically.

A second alternative would be that while starch breakdown does go as far as maltose, this maltose is never released into the metabolic pool but is metabolized as soon as it is formed. This would be analogous to the observation of Neish (6) that synthesis of xylan in wheat plants proceeds more rapidly from the xylose newly formed from glucose than from free xylose fed to cells from outside.

TABLE IV

EFFECT OF STARCH HYDROLYSIS (LEAF 1) AND SYNTHESIS (LEAF 2) ON THE SPECIFIC ACTIVITY OF MALTOSE FED TO TOBACCO LEAVES. SPECIFIC ACTIVITY OF FED MALTOSE = 0.216 μC./MGM.

Leaf No.	Maltose absorbed		Treatment after feeding	Specific activity of maltose isolated from 80% EtOH extract as μc./mgm.	Amount of starch in γ/mgm. of dry residue after extraction with 80% EtOH	Total activity in starch extracted from 1 mgm. of alcohol insoluble residue, μc.
	Mgm.	μc.				
1	17.9	3.86	One half leaf killed immediately	0.208	45.3	0.00011
			The other half of leaf attached to midrib put in darkness on water for 6 hr.	0.210	11.3	0.00006
2	11.9	2.49	One half leaf killed immediately	0.233	12.4	0.00005
			The other half of leaf attached to midrib put in light on water for 6 hr.	0.200	136.6	0.00004

Summary

1. Uniformly labelled C^{14}-maltose was fed to tobacco and wheat leaves and its metabolism was observed.

2. $C^{14}O_2$ was emitted by wheat leaves fed maltose indicating that this sugar served as a respiratory substrate.

3. Maltose fed was transformed into other carbohydrates in amounts comparable to, though somewhat lower than, glucose.

4. As in the case of the fed glucose the main carbohydrate formed from maltose was sucrose, while starch was formed in small amounts.

5. When C^{14}-maltose was introduced into tobacco leaves and left there for six hours in darkness no appreciable dilution of its specific activity was observed. In the meantime there was considerable disappearance of starch. From this it has been suggested that during the breakdown of starch *in vivo* there is no appearance of maltose in the metabolic pool of cells. Either starch breakdown does not proceed as far as maltose or the maltose formed is utilized before its release into the metabolic pool.

References

1. AHRNS, W. Weitere Untersuchungen über die Abhängigkeit des gegen-seitigen Mengenverhältnisses der Kohlenhydrate un Laubblatt vom Wassergehalt. Botan. Arch. 5 : 234-259. 1924.

2. BROWN, H. T. and MORRIS, G. H. A contribution to the chemistry and physiology of foliage leaves. J. Chem. Soc. 63 : 604-677. 1893.

3. DOUDOROFF, M., KAPLAN, N., and HASSID, W. Z. Phosphorolysis and synthesis of sucrose with a bacterial preparation. J. Biol. Chem. 148 : 67-75. 1943.

4. GIBBS, M. The position of C^{14} in sunflower leaf metabolites after exposure of leaves to short period photosynthesis and darkness in an atmosphere of CO_2. Plant Physiol. 26 : 549-555. 1951.

5. HASSID, W. Z., MCCREADY, R. M., and ROSENFELS, R. S. Determination of starch in plants. Ind. Eng. Chem. Anal. Ed. 12 : 142-144. 1940.

6. NEISH, A. C. The biosynthesis of cell wall carbohydrates. II. Formation of cellulose and xylan from labelled monosaccharides in wheat plants. Can. J. Biochem. Physiol. 33 : 658-666. 1955.

7. VITTORIO, P. V., KROTKOV, G., and REED, G. B. Synthesis of radioactive sucrose by tobacco leaves from C^{14} uniformly labelled glucose and glucose-1-phosphate. Can. J. Botany, 32 : 369-377. 1954.

OBSERVATIONS ON STEM CANKER AND POD AND STEM BLIGHT OF SOYBEANS IN ONTARIO[1]

By A. A. Hildebrand[2]

Abstract

Stem canker caused by *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora* Athow & Caldwell is a serious disease of soybeans in Ontario. Artificial inoculation experiments have indicated (*a*) that soybeans which are highly susceptible to infection at midstage in their development become progressively less so as they approach maturity, (*b*) that reduction in yield is more or less directly proportionate to earliness of infection, (*c*) that all varieties presently of commercial importance in Ontario are highly susceptible to the disease, and (*d*) that six common weeds, and tomato, pepper, and snap and field bean, are not susceptible to infection by the soybean pathogen. The tests also indicated that monoconidial isolates of the stem canker organism are as highly pathogenic as those derived from single ascospores, and that isolates, though morphologically similar, may be differentiated on the basis of their pathogenic capability. The pathogen is definitely seedborne, but infection from such a source is not of primary importance. The likelihood that the pathogen is indigenous to the soil of the soybean-growing area of Ontario is discussed. Pod and stem blight caused by *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *sojae* is of negligible economic importance in Ontario. The pathogen, which is observed mostly in the imperfect (*Phomopsis*) stage, shows little evidence of being able to infect plants until late in the growing season. In the absence of resistant varieties no better methods of control can be recommended than those of completely plowing under soybean debris, of avoiding the planting of the more susceptible varieties, and of practicing crop rotation.

Introduction

Of the 20 parasitic diseases of soybean that have been encountered in Ontario during a 10-year period, none has proved to be a more serious menace to the crop than *Diaporthe* stem canker. Even though it was also equally important in sections of North Central United States, the disease was not named until 1950 by Crall (4) in Iowa. Until then, in both Ontario and United States, stem canker had coexisted with, but had not in name at least been distinguished from, pod and stem blight, although two years earlier Welch and Gilman (21) had presented mycological and pathological evidence that would have made the distinction possible. In United States it remained for Athow and Caldwell (2) to confirm in 1954 the finding of Welch and Gilman (21) of the occurrence of two closely related but morphologically and pathologically different representatives of the genus *Diaporthe* on the soybean, and to affirm, moreover, that the diseases caused by them are quite distinct from one another.

For several years the present author has studied stem canker and pod and stem blight in southwestern Ontario where almost all of Canada's soybeans are produced. This paper reviews the scope and conduct of the studies and presents the results obtained.

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Literature Review

In North Carolina in 1920 Wolf and Lehman (23) reported *Phoma* blight as a new disease of soybeans. In 1922, Lehman (14) renamed the disease pod and stem blight and assigned *Phomopsis sojae* as the causal organism. In 1923, Lehman (15) described a species of *Diaporthe* as the cause of pod and stem blight and named it *Diaporthe sojae*. Lehman observed perithecia only in culture but three years later Wolf and Lehman (24) found them on stems that had overwintered in the field. In 1929, Sasaki (17) reported the occurrence in Korea of a *Phomopsis* disease of soybean which rendered the pods inedible. In 1933, Wehmeyer (19) in his monograph of the genus *Diaporthe*, based the taxonomy of its component species on morphological rather than on pathological considerations, and having assembled those species with elongated ostiolar necks under the general designation *D. phaseolorum* (Cke. & Ell.) Sacc., he selected the *Diaporthe* described by Harter (8) in 1917 on lima bean as the type species. He reduced *D. sojae* of Lehman on soybean, and *D. batatatis* of Harter and Field (9), which had been described on sweet potato in 1912, to varietal status, and in reference to their hosts renamed them *D. phaseolorum* var. *sojae* (Lehm.) Wehm., and *D. phaseolorum* var. *batatatis* (Harter and Field) Wehm., respectively. In 1935, Tucker (18) encountered a *Diaporthe* which caused a fruit rot of pepper and which was identified by Wehmeyer as *D. phaseolorum*. Tucker believed the fungus to be close to the variety *batatatis* of that species. By single ascospore culture he established the connection between the typical asexual (*Phomopsis*) and sexual stages and proved the homothallic character of the mycelia from single spores. In 1947, Luttrell (16) compared *D. phaseolorum* and *D. phaseolorum* var. *sojae* as to their occurrence in nature, morphology, and pathogenic capability. He postulated that positive distinction between them depends primarily upon differences in pathogenicity and appearance in culture, and that since these differences are accompanied by slight differences in morphology, *D. phaseolorum* var. *sojae* should be retained as a variety. Luttrell found perithecial and nonperithecial strains in his cultures of *D. phaseolorum* var. *sojae*. He makes no mention of having worked with single ascospore isolates. In 1948 Welch and Gilman (21), working with single ascospore isolates, differentiated between two members of the genus *Diaporthe* on soybean on the basis of sexuality, morphology, and pathogenic capability. One organism, regarded by them to be *D. phaseolorum* var. *batatatis*, was homothallic, produced its perithecia in cespitose clusters, lacked conidia, and actively attacked soybean stems, girdling them and causing plants to die; the other, recognized as *D. phaseolorum* var. *sojae*, was heterothallic, produced scattered, single perithecia and typical *Phomopsis* conidia of the alpha and beta (stylospore) types, and its parasitism was of the facultative type. As mentioned above, it was Crall (4) who in 1950 suggested that stem canker be adopted as a descriptive name for the disease of soybeans caused by *D. phaseolorum* var. *batatatis* to distinguish it from pod and stem blight caused by *D. phaseolorum* var. *sojae*. In 1954 Athow and Caldwell (2) agreed

with Welch and Gilman (21) that two morphologically different forms of *D. phaseolorum* occur on soybeans. They stated, moreover, that each of these forms is responsible for a distinct disease. They retained *D. phaseolorum* var. *sojae* (Lehm.) Wehm., as the valid name of the weakly pathogenic form causing pod and stem blight but proposed that the strongly pathogenic stem canker organism be recognized as a new variety of *D. phaseolorum* under the trinomial *D. phaseolorum* var. *caulivora*. They pointed out that the absence of a conidial or imperfect stage offers a distinct morphological basis for separating *D. phaseolorum* var. *caulivora* from *D. phaseolorum* var. *sojae*. In the latter organism they were not able to demonstrate heterothallism as Welch and Gilman (21) had done. In 1954, from five of over 1000 mature stems of soybean, the present author (11)* obtained monoascospore cultures which gave rise to *Phomopsis* isolates, the morphology of which, together with that of the perithecia on the five stems, proved the fungus to be *D. phaseolorum* var. *sojae*. The author's attempts to demonstrate heterothallism in this fungus were, like those of Athow and Caldwell (2), unsuccessful. Monoascospore cultures obtained from 20 other stems which showed a predominantly cespitose type of perithecial development gave rise to isolates of the stem canker organism. Many of those isolates reproduced only the ascus stage but a few of them revealed, in addition, the presence of conidia similar to the alpha spores of *Phomopsis*, and from which monoconidium cultures were obtained. At the time, this homothallic fungus was referred to as *D. phaseolorum* var. *batatatis* but, except for the occurrence of the imperfect stage in some isolates, it has since been found to be indistinguishable from *D. phaseolorum* var. *caulivora* as described by Athow and Caldwell (2). In September 1954, Frosheiser and Kernkamp (6) reported for the first time the inoculation of soybean plants with monoconidial isolates of *D. phaseolorum* var. *batatatis*, which they obtained in cultural studies of that organism. Late in 1954, Gerdemann (7) reported the association of a fungus presumed to be the imperfect stage of *D. phaseolorum* var. *sojae* with a root and basal stem rot of soybeans. According to Gerdemann this fungus is capable of surviving on soybean stems in the soil for long periods of time.

Symptomatology

Stem Canker

Sometimes late in July but more often towards the middle of August attention is attracted to occasional plants whose dead tips render them conspicuous in many stands of soybeans in southwestern Ontario. Examined closely, these plants are found to be wilting and dying, or already dead, and the cause of their condition can almost invariably be attributed to a girdling type of lesion (Fig. 1) which is present on the lower half of the stem. The lesion usually occurs at either a leaf scar (Fig. 9) or a tear or break at the juncture of a branch and a main stem. A reddish-brown discoloration is usually a distinctive feature of lesions in their earlier stages. This discoloration

**This paper was in press when the article by Athow and Caldwell (2) appeared in print.*

may extend over the whole surface of the lesion, or occurring marginally, may border a central grayish-brown area. In any case, it delimits lesions sharply (Figs. 1 and 9). Lesions quickly girdle the stem, and the tissue invaded by the causal fungus soon becomes so dry and brittle that the stem will readily snap at the point of attack. As the lesions become older, the reddish-brown discoloration gives place to shades of brown that range from light to almost chocolate. On some stems, old lesions are eventually almost black; on others, they show a purplish tinge. In extent they range from one to several inches (Fig. 8). On and near old lesions, small stromatal bodies superficially resembling pycnidia often appear more or less abundantly. The present author has already referred to these bodies as "stromatal islands", and expressed the opinion that they are precursors of either pycnidia or perithecia (11).

From mid-August until the end of the growing season, increasing numbers of plants show the symptoms described above, and in most seasons the disease becomes epiphytotic on the more susceptible varieties.

Occasionally, late in the growing season, perithecia have been found on plants, not on lesions such as those described above, but on diseased areas low on the stem near the ground level. These perithecia belonged to the stem canker organism. The disease as it occurs on mature stems has already been dealt with in detail by the author (11).

Pod and Stem Blight

In general, symptoms truly diagnostic of pod and stem blight do not become readily apparent until after an infected plant has become mature. Then, however, they are quite prominent, especially on the stem where they appear as large numbers of small black bodies which tend to be disposed linearly (Fig. 5). On some infected areas these small black bodies are typical mature pycnidia; on others they are predominantly stromatal in nature. Often pycnidia will be found at one extremity of an infected area, and "stromatal islands" at the other, while intervening is a transitional area where the one form shades into the other. Fruit bodies or their precursors have also been found on pods (Fig. 4), though much less frequently than on stems.

Complicacy in Symptoms of the Two Diseases

The symptomatology of the two diseases is not as distinct as the above descriptions would suggest. If plants are examined carefully early in August, large numbers of dead or dying spurs and petioles (Figs. 2 and 3) will be noted on the lower regions of the stems. On most of these affected spurs or petioles

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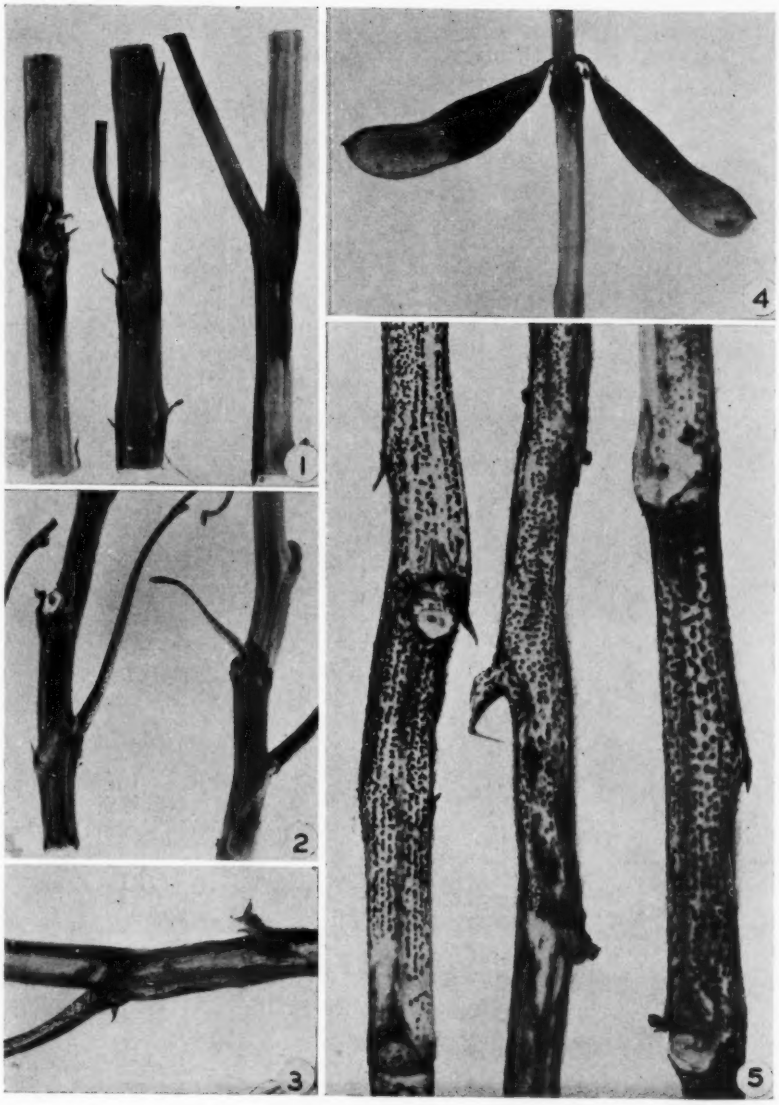
FIGS. 1–5. Symptoms of *Diaporthe* stem canker and pod and stem blight on soybean. FIG. 1. Girdling type of stem canker lesions on stems. FIG. 2. Petiole (left) infected with pod and stem blight (note fruiting bodies) and spur (right) killed by stem canker. FIG. 3. Pod and stem blight (lower left) and stem canker (upper right) occurring in close proximity on same stem. FIG. 4. Pod and stem blight on pods. FIG. 5. Pod and stem blight on stems resulting from artificial inoculation by toothpick method.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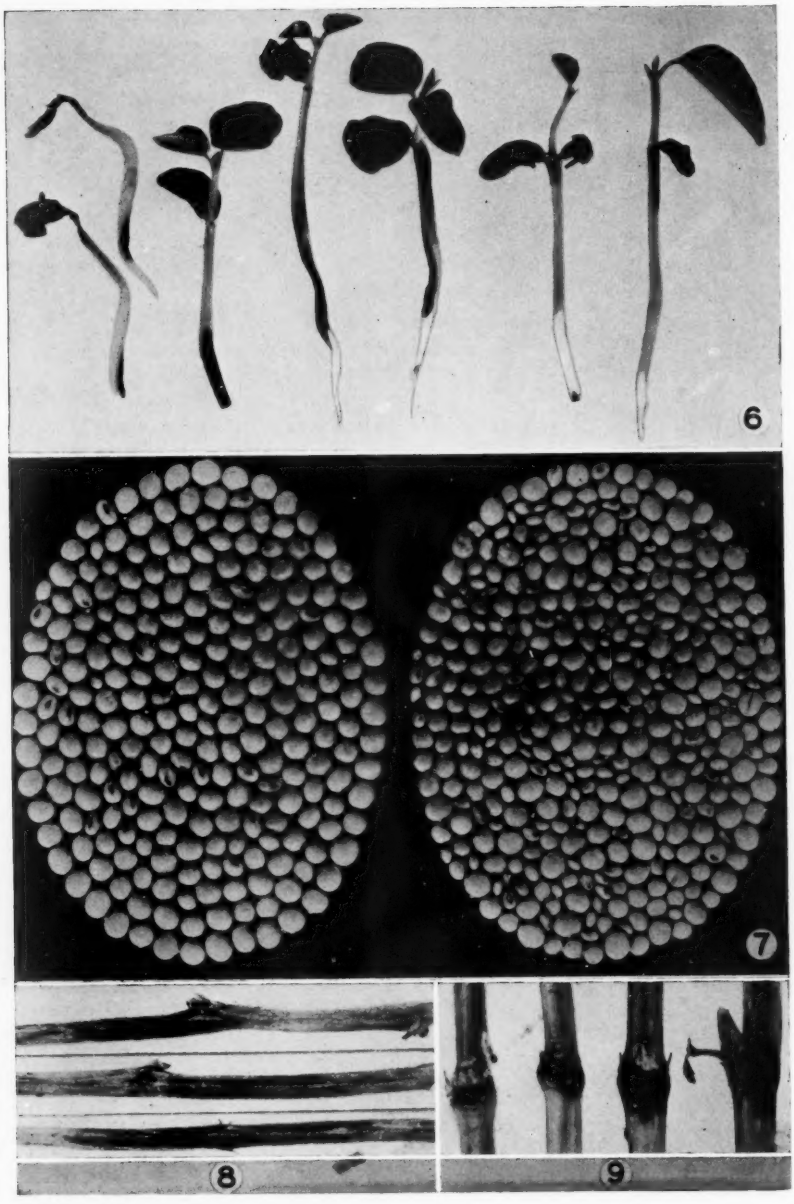

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necrosis that started at or towards the tip has spread for varying distances back toward the main stem. As the season advances, many of the diseased spurs and petioles (particularly the latter) drop off, but from those that remain attached infection often spreads into the main stem and lesions develop (Figs. 2 and 3). A cursory examination will reveal the presence of fruit bodies on some of the affected spurs and petioles but not on others. Of 520 affected spurs and petioles collected at random from Lincoln plants in a commercial stand on August 8, 1954, and later examined critically in the laboratory, 44.6% showed pycnidia and "stromatal islands", 13.1% perithecia and "stromatal islands", and the remaining 42.3% lacked visible evidence of fruit bodies of any kind. From pycnidium-bearing spurs and petioles were obtained cultures of *Phomopsis*, some of which were morphologically indistinguishable from imperfect stage representatives of the pod and stem blight fungus. Perithecium-bearing spurs and petioles consistently yielded cultures of the stem canker fungus. Most of the spurs and petioles which showed necrotic tissue but lacked fruit bodies yielded cultures of the stem canker fungus, but from 24 of the 220 in this category, the pod and stem blight organism was obtained. The two fungi were never isolated from the same spur or petiole but they have frequently been isolated from distinctive pod-and-stem-blight and stem-canker areas of infection only a short distance apart on the same stem (Fig. 3).

Athow and Caldwell (2) noted in their study of the two diseases that pod and stem blight first appears on the petioles of lower leaves and upon broken branches but they made no mention of having observed stem-canker-infected spurs and petioles.

Isolations

Methods

Isolations have been made from many specimens of soybeans selected discriminatively and differing as to age, stage and severity of infection, variety, and location obtained. Transfers of spores from pycnidia and perithecia, of "stromatal islands", and of particles of infected tissue from stems, spurs, and petioles to 2% dextrose natural potato agar, acidified to the extent of one drop of 10% lactic acid per ml., have readily yielded cultures of the fungi associated with stem canker and pod and stem blight. All isolates intended for future use were purified by the monospore method. Some isolates did not form fruit bodies until grown on steamed soybean stems or on natural potato agar with reduced dextrose content.

FIG. 6. Fifteen-day-old soybean seedlings, variety Blackhawk, grown in soil infested with *Phomopsis* sp(p). (the three on right) and with *D. phaseolorum* var. *caulivora* (the four on left). Note cotyledonary infections and lesions on and at base of the hypocotyl. FIG. 7. Difference between seed from healthy (left) and from stem-canker-infected (right) mature soybean plants, variety A. K. Harrow. FIG. 8. Stem canker lesions on mature stems of plants killed by the disease. FIG. 9. Early stages of leaf scar infection by *D. phaseolorum* var. *caulivora*.

Categories of Isolates

The isolates which were regarded as significant to the present studies fell into three categories as follows:

(*a*) *Phomopsis* sp(p). Because *Phomopsis* develops in nature on the stems of many weed and cultivated plants, including the soybean, after they have become mature, it would seem that some representatives of the genus might be primarily saprophytic. Most attempts to isolate *Phomopsis* were made, therefore, before the normal maturity date of any of the soybean varieties concerned, and from plants which showed lesions or necrotic areas on spurs, petioles, or stems that in appearance at least were suggestive of recent attack by a parasite. Altogether 22 isolates of *Phomopsis* were obtained, one as early as July 27, several early in August, and none later than September 14. In general, infected areas from which *Phomopsis* isolates were obtained did not show the reddish-brown discoloration that is almost always a distinguishing characteristic of the stem canker lesion in its earlier stages.

(*b*) *D. phaseolorum* var. *sojae*. As mentioned above in the review of literature, and as described in detail in a recent publication (11), five isolates of *D. phaseolorum* var. *sojae* were obtained by the writer from mature stems of soybeans. By single ascospore culture the genetic connection with the typical asexual (*Phomopsis*) stage was established.

(*c*) *D. phaseolorum* var. *caulivora*. This fungus was isolated as early as the last week in July from spurs and petioles which showed necrotic tissue alone or necrotic tissue and perithecia. Many isolates were obtained during August and later from stem lesions developing from leaf-scar infections. From cespitosely-grouped perithecia on mature stems that had either overwintered in the field or been placed in water in the greenhouse (11), isolates were also obtained. A number of those from the latter source produced the imperfect stage and it was possible to make monoconidium as well as monoascospore cultures. In a few instances isolates were obtained before the end of the growing season from cespitose perithecia near the base of the stem of affected plants.

Pathogenicity Trials

Preliminary Tests

With the above-mentioned isolates, many pathogenicity trials were carried out both in the greenhouse and in the field earlier in the investigations. Because of incorrect timing of inoculation in relation to age of plants, or failure to provide the isolate under test with an infection court in which it could establish itself, the results of a considerable number of the earlier trials were inconclusive or misleading. Gradually, however, a timing and technique were evolved which proved to be more consistently reliable and satisfactory. For the inoculating of plants no better method was found than the elaboration by the author (10) of Crall's "toothpick" method (5), provided that the incision in the stem, in which the truncated toothpick is embedded, is thoroughly sealed with vaseline. It was further discovered that plants should be inoculated earlier rather than later in their developmental stage, that is,

when they are between 60 and 70 days old, and that field populations are better for the purpose than plants grown in the greenhouse. Uniformity of growth of plants to be inoculated was most satisfactorily attained by early-season hand-thinning which left seedlings evenly spaced at 4-in. intervals in the rows.

In general, the results of the earlier series of trials led definitely to the conclusion that isolates of *Phomopsis* and of *D. phaseolorum* var. *sojae* were, at most, only weakly pathogenic, whereas those of *D. phaseolorum* var. *caulivora* were with only few exceptions strongly pathogenic to plants of the most important commercial varieties. Certain of the trials, especially those involving *D. phaseolorum* var. *caulivora*, were duplicated on soils of widely differing type, that is, a Fox sandy loam at Harrow, a Brookston clay at the Woodslee substation of the Harrow Experimental Station. No difference in the pathogenicity of isolates to plants grown on these different soil types was ever noted.

Major Tests

During the summer of 1954, a pathogenicity trial culminant to many which had preceded was carried out in the laboratory experimental plots. The experiment was also designed to explore more fully the apparent correlation between age of plants and severity of attack by the pathogen. On July 19, August 3, and August 17, when Blackhawk plants were 55, 71, and 83 days old, respectively, they were inoculated by the toothpick method with 10 different isolates, five of *D. phaseolorum* var. *sojae* (designated by the numerals 1, 2, 3, 4, and 5), and five of *D. phaseolorum* var. *caulivora* (designated by the numerals 6, 7, 8, 9, and 10). Each isolate of *D. phaseolorum* var. *sojae*, originally of monoascospore origin, had produced the asexual or *Phomopsis* stage, monoconidium subcultures of which provided the inoculum for the experiment being described. Each isolate of *D. phaseolorum* var. *caulivora* was also of monoascospore origin. Isolates 6, 7, and 8 had not only reproduced the ascus stage but had also given rise to a *Phomopsis*-like imperfect stage from which monoconidium cultures were obtained. Thus, opportunity was afforded of testing the pathogenicity of eight different isolates, five of *D. phaseolorum* var. *sojae* and three of *D. phaseolorum* var. *caulivora*, all of immediate monoconidium origin. So far as the writer is aware such a comparison has not been possible previously. An imperfect stage of *D. phaseolorum* var. *caulivora* isolates 9 and 10 was never found. On July 26, August 9, and August 23, when Lincoln plants were 62, 76, and 91 days old, respectively, they were inoculated in the same way with the same isolates. The inoculations were begun earlier with Blackhawk because that variety matures earlier than Lincoln. On each date, 25 pairs of plants of each variety were selected at random. One plant of each pair was inoculated with a particular isolate, while the companion plant, into which was inserted a sterile toothpick, served as check. The total number of plants involved in the experiment is indicated as follows:

2 (varieties) × 3 (dates) × 10 (isolates) × (25 inoc. + 25 ck. plts.) = 3000.

On the 15th day after inoculation, and at five-day intervals thereafter, the plants were examined macroscopically and their symptoms recorded. The results of these examinations are presented graphically in Figs. 10 and 11. In these two figures it will be noted that nothing is depicted for checks, the reason being that the 1500 check plants remained healthy virtually to the extent of 100%. A few were lost by accident but none by natural infection.

Figs. 10 and 11 show that the five isolates of *D. phaseolorum* var. *sojae* were virtually nonpathogenic to 62- and 76-day-old Lincoln, and 55- and 71-day-old Blackhawk plants. It was not until 50 days after inoculation that a few plants of both varieties in the age groups specified began to show slight evidence of infection. Inferentially then, *D. phaseolorum* var. *sojae* does not become pathogenic until the host almost reaches maturity. This likelihood is strengthened by the fact that isolates 1 and 2 of *D. phaseolorum* var. *sojae* were able within 30 days to attack a few of the Blackhawk plants that had been inoculated when they were 83 days old but not within 30 days any of those of the same variety that had been inoculated when they were 71 or 55 days old. Plants of both Blackhawk and Lincoln that were finally parasitized by isolates of *D. phaseolorum* var. *sojae* showed at maturity typical symptoms of pod and stem blight such as are shown in Fig. 5.

In marked contrast, all isolates of *D. phaseolorum* var. *caulivora*, whether of monoascospore or of monoconidium origin, were strongly pathogenic, especially to the earlier-inoculated plants of both varieties. As Fig. 10 shows, only 15 days after inoculation, mortality among 62-day-old Lincoln plants was extremely high, ranging from about 67% for isolate 8 to about 90% for isolate 9. By 30 days after inoculation destruction of Lincoln plants in this age group was almost complete for all isolates. As Fig. 11 shows, destruction of Blackhawk plants, inoculated when they were 55 days old, was slower but eventually almost as complete as that of the earliest-inoculated Lincoln plants. Reference again to Fig. 10 will show that Lincoln plants, inoculated when they were 76 and 91 days old, did not become infected as soon as those that were inoculated when they were 62 days old; nor was destruction among the former as complete as among the latter. The inference is that as plants of the variety Lincoln become older, they are less readily susceptible to infection and killing by the stem-canker-inducing organism. With regard to Blackhawk, Fig. 11 shows that there was a delay in infection of 83-day-old plants but that by the 30th day after inoculation mortality was almost as high as among earlier-inoculated plants. These findings are not in accord with those of certain workers elsewhere. In Indiana, for example, the data of Athow and Caldwell (2) suggested that older plants of the variety Hawkeye were more susceptible than younger ones to a number of isolates of *D. phaseolorum* var. *caulivora*. A comparison of Fig. 10 with Fig. 11 reveals that in the aggregate Blackhawk was more susceptible than Lincoln to stem canker.

In the experiment under consideration isolate 9 of *D. phaseolorum* var. *caulivora* attracted attention. Figs. 10 and 11 show that this isolate was strongly pathogenic to 62-day-old Lincoln and only slightly less so to 55- and

FIGS. 10 and 11. Results of inoculating soybeans of three different age groups, varieties Lincoln (FIG. 10) and Blackhawk (FIG. 11), with five isolates of *D. phaseolorum* var. *sojae*, and five of *D. phaseolorum* var. *caulivora*. All cultures were originally of monoascospore origin but isolates 1 to 8 produced the asexual stage and it was possible with them to use cultures of monoconidium origin.

71-day-old Blackhawk plants. The isolate seemed almost incapable, however, of infecting 76- and 91-day-old Lincoln, and 83-day-old Blackhawk plants. This circumstance implied some change in either the fungus or the host. In December 1954, an isolate-9 subculture of the one that had been used in the field experiment was used to inoculate twenty 54-day-old Blackhawk and twenty 62-day-old Lincoln plants in the greenhouse. In 15 days, all the inoculated plants of both varieties were dead, while all the check plants remained in a healthy condition. Thus, the fungus had not lost its pathogenicity, and its failure to infect older plants in the field, as indicated above, would seem to be accountable to some change in the plant that rendered it less susceptible to this particular isolate. The fact that isolates 6, 7, 8, and 10 could still infect and kill older Blackhawk and Lincoln plants would seem to differentiate them from isolate 9 on the basis of pathogenic capability. Athow and Caldwell (2) found no appreciable difference in pathogenicity among their isolates of *D. phaseolorum* var. *caulivora*. Isolate 9 could not be differentiated from isolates 6, 7, 8, and 10, culturally or morphologically.

To try to confirm quantitatively the differences observed macroscopically, as described above, all of the Lincoln plants included in the three different age groups were harvested in pairs, and the yields of the 25 inoculated plants comprising a test unit were compared with those of the 25 complementary

TABLE I

EFFECT OF INOCULATION OF SOYBEAN PLANTS, VARIETY LINCOLN, OF THREE AGE GROUPS, WITH FIVE ISOLATES OF *D. phaseolorum* VAR. *caulivora*, AS INDICATED BY THE DIFFERENCE BETWEEN THE MEANS OF THE YIELD OF INOCULATED AND NONINOCULATED PLANTS

Age of plants (days)	Isolate designation	Mean difference in favor of noninoculated plants, gm.	Degrees of freedom[a]	Observed value of t	Value of t required for significance	
					.05	.01
62	6	29.15[b]	23			
	7	33.98	24			
	8	32.67	24			
	9	33.69	23			
	10	33.09	23			
76	6	15.83	23	5.380**	2.069	2.807
	7	12.43	24	3.743**	2.064	2.492
	8	20.06	24	6.965**	2.064	2.492
	9	3.84	21	2.461*	2.080	2.831
	10	12.70	23	5.594**	2.069	2.807
91	6	5.27	24	2.803**	2.064	2.492
	7	3.49	23	2.280*	2.069	2.807
	8	4.36	23	3.516**	2.069	2.807
	9	1.43	22	1.152	2.074	2.819
	10	2.33	24	1.181	2.064	2.492

[a]*Slight difference in degrees of freedom arose from loss of occasional plants by breakage or some other extraneous circumstance.*

[b]*As almost all of the 62-day-old plants had been killed, there was virtually no yield from them; thus, the difference shown may be regarded as significant without recourse to analysis.*

*, **Indicate values of t exceeding the 5 and the 1% level of significance, respectively.

checks. The difference between the means of yield of inoculated and of check plants was tested for significance by application of "Student" *t* test, with results as shown in Table I.

As Table I shows, the difference between the means of yield of 25 inoculated and of 25 complementary, noninoculated Lincoln plants was (*a*) always in favor of the noninoculated plants, and (*b*) greatest, intermediate, and least for 62-, 76-, and 91-day-old plants, respectively, the latter circumstance indicating a diminishing severity of attack with increasing age of plants. Thus, the evidence of a quantitative nature confirmed that obtained by critical macroscopic examination, and illustrated graphically in Figs. 10 and 11. Among plants of the 76-, and the 91-day age groups, the mean difference in favor of noninoculated plants was least in the series in which isolate 9 of *D. phaseolorum* var. *caulivora* was the organism involved. This evidence of a quantitative nature supports the inference that isolates of *D. phaseolorum* var. *caulivora* may differ as to their pathogenic capability.

Studies as to the Seed- or Soil-borne Nature of Stem Canker and Pod and Stem Blight

In 1949, stem canker, which up to that time had not attracted serious attention, suddenly became of outstanding importance in Ontario. That year, in anticipation of a greatly increased acreage, and because of a shortage in the domestic supply, large quantities of seed, particularly of the variety Hawkeye, were imported into the province and widely distributed for planting. By late August severe outbreaks of stem canker had occurred in many Hawkeye plantings. In 1950, the severity of the disease was again outstanding on Hawkeye. In 1951, in addition to Hawkeye, the variety Blackhawk was planted fairly extensively in Ontario. Blackhawk proved to be even more susceptible than Hawkeye. The opinion prevailed that the disease had been introduced with the seed of the two varieties. The situation in general led to the following studies.

Isolations from Seed

In September 1949, when an unmistakably more severe disease pattern had been established for Hawkeye than for other varieties, small samples of seed of the variety left over from spring planting were obtained from two growers in whose stands stem canker was severe. Leftover seed of the variety Harman was also obtained from another grower in the same district, in whose stand stem-canker-infected plants were present to the extent of about 1.5%. Macroscopically, there was nothing to suggest the presence of disease in these three lots of seed. One hundred seeds from each lot were assembled in cheesecloth bags and surface sterilized by immersion in a solution of sodium hypochlorite (Clorox), in accordance with the technique described by Wells *et al.* (22) in their study of near-wilt of pea. The seeds were then placed on natural p.d.a. (2% dextrose) in Petri plates, five seeds per plate, which were incubated at room temperature. As soon as fungus growth could be detected, transfer of mycelium was made to p.d.a. slants, from which, later, identifications were made.

The results of the isolations were surprising. Twelve seeds of the variety Harman, which has always shown a low incidence of stem canker in the field in Ontario, yielded cultures of *D. phaseolorum* var. *caulivora*, whereas only eight and seven seeds of the respective lots of Hawkeye yielded cultures of the pathogen. The seeming anomaly of these results has been repeated many times in regard not only to varieties that give strong evidence in the field of differing in their susceptibility to the disease but also to stands of plants in which the number of infected plants has varied widely. Of interest in this connection are the recent findings of Kilpatrick and Hartig (13) who report that they obtained 13 different fungi from apparently healthy, surface-sterilized seed of the variety Ogden, the purple stain fungus *Cercospora kikuchii*, and *Phomopsis* being the most frequently isolated.

Result of Planting of Seed from Healthy and from Stem-Canker-Infected Plants

On September 5, 1952, a commercial stand of soybeans, variety A. K. Harrow, was encountered in which 42% of the plants were found to be dead or dying as the result of attack by stem canker. Seed was harvested by hand from plants which had been killed by the disease and all of which showed stem lesions like those shown in Fig. 8. Seed was also obtained from healthy plants in the stand. Fig. 7 shows seed from healthy (left) and from diseased (right) plants. A portion of the seed from diseased plants was treated with Spergon at 3 oz. per bu., another with Arasan at 2 oz. per bu., while a third was left untreated. The seed from healthy plants was left untreated.

On May 15, 1953, seed of each lot was planted by hand in plots at the laboratory in a randomized, five-replicate design, the test unit comprising rows 30 ft. long. On the site chosen, soybeans had never been grown previously. On June 11, 28 days after planting, counts of plants were taken, a distinction being made between healthy plants and those that appeared abnormal in any way. In August and September, stem-canker-infected plants were counted. On October 18, plants were harvested and threshed and yields were determined. The results of these various operations are summarized in Table II.

TABLE II

STAND OF PLANTS, INCIDENCE OF STEM CANKER, AND YIELD RESULTING FROM PLANTING OF SEED FROM HEALTHY AND FROM STEM-CANKER-INFECTED A. K. HARROW PLANTS

Source of seed	Treatment of seed	Stand of plants, %			Incidence of stem canker, %	Yield, bu. per ac.
		Healthy	Abnormal	Total		
Healthy plants		74.5	7.2	81.7	6.1	28.29
Diseased plants	Spergon	66.2	6.8	73.0	5.9	28.37
" "	Arasan	68.0	5.1	73.1	8.5	27.80
" "		67.1	6.0	73.1	6.8	28.90

Table II shows that seed from healthy plants gave a stand about 8% higher than that from seed obtained from diseased plants. This 8% increase in stand is not reflected, however, in a corresponding increase in yield. The yield, and the incidence of plants infected with stem canker, was almost the same regardless of the source or treatment of seed. The results do not suggest that dissemination of the disease through the seed is important.

Seedling Infection in Artificially-infested Soils

Although circumstantial evidence suggested that the serious outbreaks of stem canker in Ontario on the varieties Hawkeye and Blackhawk in 1949 and succeeding years had their origin in seed-borne inoculum, nevertheless there remained the possibility that the pathogen was indigenous to the soil of the affected areas. Consideration of this possibility led to a series of trials which centered around the artificial infestation of soils with organisms isolated from both stem-canker-infected and pod-and-stem-blight-infected plants. The most important of these trials is described below.

On February 2, 1952, sterilized rye grain in Erlenmeyer flasks was inoculated with nine randomly selected Ontario isolates of *D. phaseolorum* var. *caulivora*, an isolate of the same organism obtained from Kirk L. Athow in Indiana, and seven isolates of *Phomopsis* sp(p). One hundred and eight 8-in. pots were filled with a Fox sandy-loam field soil, obtained from a location where soybeans had never been grown. On February 14, 17 groups comprising six pots each, were inoculated with the 17 different isolates, by incorporating 30 gm. of inoculum with the top two inches of soil in each pot. Three pots of each six-pot group were planted to Harman, the other three to Blackhawk, 15 seeds, untreated but selected for freedom from disease, being planted in each pot. As checks, similar plantings were made in pots in which noninoculated sterilized rye grain was added to the soil. The pots were randomized in the greenhouse and they were watered uniformly. During the experiment the temperature ranged from 18° to 21° C. On March 1, 15 days after the seed had been planted, records of emergence were made. For two additional weeks records were made also of incidence and severity of disease among emerged seedlings. The results are recorded in Table III.

As Table III shows, 9 of the 10 isolates of *D. phaseolorum* var. *caulivora*, including the Indiana isolate, caused significant reductions in emergence of seedlings of both varieties, as compared with check. Isolate 3 affected emergence only slightly. This apparent difference in pathogenicity between isolate 3 and the others supports the probability of the existence of different strains of the stem canker fungus.

Only three of the seven *Phomopsis* isolates reduced emergence significantly in the two varieties. Thus, in accordance with previous evidence, representatives of the genus *Phomposis* are not, in general, as important in the pathology of the soybean as those of *D. phaseolorum* var. *caulivora*. That *Phomopsis* isolates may, however, possess outstanding pathogenic capability under certain conditions is shown by the severity of the attack of isolates I and II on the

TABLE III

EMERGENCE OF, AND POSTEMERGENCE INFECTION AND MORTALITY AMONG, SEEDLINGS OF TWO VARIETIES OF SOYBEANS GROWN IN SOIL ARTIFICIALLY INFESTED WITH ISOLATES OF *D. phaseolorum* VAR. *caulivora* AND OF *Phomopsis* SP(P).

Organism	Isolate designation	Harman					Blackhawk				
		Emergence				Postem. infection and mortality, %	Emergence				Postem. infection and mortality, %
		Replicates			Transformed means $\sqrt{x+0.5}$		Replicates			Transformed means $\sqrt{x+0.5}$	
		1	2	3			1	2	3		
Check		14	15	13	3.8063	—	15	15	15	3.9370	—
Diaporthe phaseolorum var. *caulivora*	1	0	0	0	0.2236**	—	1	1	3	1.4400**	11.1
	2	2	2	1	1.4623**	—	2	3	1	1.5588**	8.8
	3	15	14	14	3.8508	—	14	15	14	3.8508	—
	4	2	8	8	2.4706**	8.8	8	7	11	3.0150**	11.1
	5	3	2	4	1.8577**	15.5	4	0	7	1.6945**	13.3
	6	7	6	10	2.8428**	8.8	5	8	7	2.6664**	13.3
	7	3	5	5	2.1870**	6.6	10	6	8	2.9017**	17.7
	8	8	6	8	2.7934**	13.3	8	9	12	3.1777**	11.1
	9	3	3	9	2.2746**	17.7	8	11	11	3.2325**	15.5
	Indiana	10	6	7	2.8428**	12.3	7	5	8	2.6664**	8.7
Phomopsis sp(p).	I	5	4	0	1.5633**	2.2	0	0	1	0.5573**	6.4
	II	8	7	9	2.9120**	2.2	5	5	11	2.6938**	8.8
	III	12	14	13	3.7187	4.4	15	14	15	3.8939	—
	IV	15	12	10	3.6171	4.4	15	12	12	3.6693*	4.4
	V	11	11	8	3.2325**	6.6	15	13	13	3.7618	—
	VI	12	10	12	3.5295	—	15	15	15	3.9370	—
	VII	14	13	12	3.8063	2.2	15	15	15	3.9370	—
		L.S.D. 0.05 = 0.298 0.01 = 0.399					L.S.D. 0.05 = 0.235 0.01 = 0.315				

*, **Significant at 0.05 and 0.01 levels, respectively.

seeds of both varieties. These two isolates may represent a strain or strains differing pathogenically from other possible strains represented by isolates III, VI, and VII.

Infection and mortality carried over into the postemergence phase of development of the seedlings. As Table III shows, the number of seedlings that showed a diseased condition after emerging from soil infected with *D. phaseolorum* var. *caulivora* reached a maximum of 17.7% for both Harman (isolate 9) and Blackhawk (isolate 7). In contrast, the maximum for diseased seedlings growing in *Phomopsis*-infested soil reached only 8.8% (var. Blackhawk, isolate II). In Fig. 6 are shown seven 15-day-old Blackhawk seedlings, the three on the right being from *Phomopsis*-infested soil, and the four on the left from soil infested with *D. phaseolorum* var. *caulivora*. They exhibit three main types of injury. In one, the cotyledons are attacked, and from them infection often spreads into the tissues of the stem. Thirty-three years ago, Lehman (14) noted that the fungus, to which at the time he ascribed the name *Phomopsis sojae*, caused the death of seedling soybean plants by growing from the seed coat into the hypocotyl and causing its decay. In a second type of injury, lesions variously occurring on the hypocotyl mark points of attack by the pathogen concerned. The third and most commonly occurring type of injury was a rotting of the roots and basal portion of the stem. Recently Gerdemann (7), working with a pycnidial fungus that was presumably the imperfect stage of *D. phaseolorum* var. *sojae*, observed this latter type of injury. Often, early in the growing season in Ontario, diseased seedlings are observed in many commercial stands of soybeans. The question arises as to how many of these may have been attacked by the stem-canker-, or the pod-and-stem-blight-inducing organism.

By the usual cultural methods, several of the isolates with which soils had been respectively inoculated were recovered from lesions on the hypocotyls of seedlings grown in those particular soils. The isolates could not, however, be recovered from healthy-appearing tissue of the hypocotyls, and there was no evidence that the seedlings had become systemically infected.

The results of the experiments described in the three preceding sections may be recapitulated as follows:

(*a*) The stem canker organism is definitely seed-borne but not to a degree nor with a consistency that seems relative either to extent of field infection of plants, nor to apparent differences in susceptibility among varieties.

(*b*) Although seed obtained from healthy A. K. Harrow plants, and from stem-canker-infected plants of the same population, gave a difference in stand that was appreciably greater for the former, nevertheless, yield from the two lots of seed was about the same, and the incidence of plants infected with stem canker was also about the same.

(*c*) When seeds of an apparently resistant variety (Harman) and of a susceptible variety (Blackhawk) were planted in soils artificially infested with isolates of the stem canker and the pod and stem blight organisms, respectively, certain of the isolates of each organism not only caused significant reductions

in emergence of seedlings of the two varieties but also infected seedlings in the postemergence phase of their development, the most common type of injury being a rotting of the roots and basal portion of the stem.

Although the foregoing results do not directly answer the question as to whether the pathogen which caused the most serious epiphytotics of stem canker in Ontario in 1949, 1950, and 1951 was introduced with imported seed or was already in the soil, nevertheless they contribute to a consideration of the probabilities of the situation. It is not unlikely that at least as high as 8% of the seed of Hawkeye and of Blackhawk imported into Canada during the period in question may have carried the stem canker organism. Such an inoculum potential, though appreciable, might still have been only contributory and of secondary importance to one already existent in the soil. For a number of years previous to 1949 and the advent of Hawkeye seed, varieties including Lincoln, Harman, A. K. Harrow, Richland, Earlyana, and others had been grown in the area under consideration. Stem canker (then not differentiated from pod and stem blight) had been noted on all those varieties, in some instances the disease having been fairly severe on Lincoln, A. K. Harrow, and Earlyana. Thus, there was the probability not only of widespread infestation of soil but also of build-up of inoculum in it, before the first large-scale plantings of the extremely susceptible Hawkeye and Blackhawk varieties in 1949 and succeeding years.

It was recorded above that in a given lot of Harman seed, 12% of the seed was infected with the stem canker organism; yet, in many years of observation, the incidence of stem canker in the field in Harman has never approached that figure. It was shown also that there was negligible difference in the incidence of stem canker among plants of the variety A. K. Harrow that had originated on the one hand from plants that had been killed by the disease, and on the other from healthy plants of the same population. These circumstances seem to point to a soil-borne rather than a seed-borne inoculum as primarily responsible for the incidence of stem canker in Ontario. In 1952 Chamberlain (3) commented that seed transmission does not seem to be the most important mode of dissemination for stem canker under field conditions in Iowa, and in 1954 Athow (1) suggested that the widespread growing of the very susceptible variety Hawkeye had caused the build-up of stem canker in Indiana.

Effect of Stem Canker and Pod and Stem Blight on Quality and Yield of Seed

Pod and stem blight is virtually of negligible economic importance in Ontario. Stem canker, on the other hand, impairs quality of seed and reduces yield. How seed may be impaired by the disease is shown in Fig. 7. Earlier in the present paper it was stated that the seed to the left in Fig. 7 was obtained in September 1952 from mature healthy A. K. Harrow plants, while that to the right was obtained at the same time from plants of the same population that had been killed by the disease. The seeds from the healthy plants shown in the left oval number 239, of which 94.9% are of normal size for the variety.

In the right oval the seeds from the plants killed by the disease number 367, of which only 58.5% are of normal size. The average weight of 1000 seeds from the healthy plants is 158.32 gm., while that of the same number of seeds from diseased plants is 99.02 gm. Thus, the disease in this particular stand of soybeans caused an approximate 37% reduction in seed weight. This figure does not take into account impairment in quality or the amount of small, shrivelled seed that would be lost in commercial threshing and cleaning operations. Every year, stem canker is unmistakably evident in many hundreds of soybean fields in Ontario. The seed harvested from affected fields must show, to a degree that varies with the time and extent of infection, deleterious effects similar to those described above.

Stem canker is of further importance economically because it attacks and kills actively growing plants. The extent of loss depends upon the stage of development of the plant at the time of infection. In an attempt to gain some information about loss of yield in relation to date of infection a fairly large-scale experiment was carried out during the summer of 1954.

On August 18, 85-day-old plants of the variety Lincoln, growing in a laboratory experimental plot, were inoculated by the toothpick method with two isolates each of *Phomopsis* sp(p)., *D. phaseolorum* var. *sojae*, and *D. phaseolorum* var. *caulivora*. The date of inoculation was chosen to coincide with the time when stem canker usually begins to attract attention in commercial plantings. The plots which had been planted on May 25 had been thinned by hand on June 14 with the result that when the inoculations were carried out the plants were growing evenly in the rows about four inches apart. Thirty successive plants were inoculated with each organism in a randomized, five-replicate design. Sterilized toothpicks were inserted into the stems of plants that were to serve as checks. Throughout the experiment natural infection was not a complicating factor. The plants were harvested and threshed on October 25, and yields were determined by immediate weighings. The results are recorded in Table IV.

TABLE IV

COMPARATIVE YIELDS FROM SOYBEAN PLANTS, VARIETY LINCOLN, INOCULATED AUGUST 18, 1954, WITH ISOLATES OF *Phomopsis* SP(P)., *D. phaseolorum* VAR. *sojae*, AND *D. phaseolorum* VAR. *caulivora*

Organism	Isolate	Replicates 1	2	3	4	5	Totals, oz.	Means, oz.
		Yield, oz.						
Check	—	39.1	31.7	35.7	39.5	36.6	182.6	36.5
D. phaseolorum var. *sojae*	3	33.0	31.7	42.0	33.9	42.6	183.2	36.6
" " "	2	34.8	35.0	36.8	38.2	36.3	181.1	36.2
D. phaseolorum var. *caulivora*	10	28.2	21.2	26.8	34.8	28.9	139.9	27.9**
" " "	6	37.8	28.5	28.4	19.3	32.6	146.6	29.3*
Phomopsis sp.	11	37.3	31.7	33.1	38.8	42.5	183.4	36.6
" "	12	36.7	27.2	31.9	45.9	38.7	180.4	36.0
		L.S.D. 0.05 = 6.4; 0.01 = 8.7						

*, ***Significant at 0.05 and 0.01 levels, respectively.***

As Table IV shows, the yield from check plants and from those inoculated with either *D. phaseolorum* var. *sojae* or the *Phomopsis* isolates were identical. In contrast, the yields of plants inoculated with each of the isolates of *D. phaseolorum* var. *caulivora* were significantly less than that of check, the reduction for isolate 10 amounting to 23.6%, and for isolate 6, to 19.8%. These figures do not take into account seed of poor quality or the fraction of undersized seed that would be lost in commercial threshing and cleaning practice.

Isolates 6 and 10 of *D. phaseolorum* var. *caulivora* were used in the major pathogenicity trials described in an earlier section of this paper to inoculate 62-, 76-, and 91-day-old Lincoln plants. If differences in yield between check and inoculated plants in those trials are computed and listed with those of the experiment just described, the percentage reduction in yield can be related to age of plants inoculated at four different developmental stages with the two isolates in question. Table V shows this relationship.

Table V shows how closely, under conditions of artificial inoculation at least, loss of yield may be related to plant maturity at time of infection. The data also suggest a difference between the two isolates in pathogenic capability.

TABLE V

PERCENTAGE REDUCTION IN YIELD IN RELATION TO AGE OF SOYBEAN PLANTS, VARIETY LINCOLN, INOCULATED WITH TWO ISOLATES OF *D. phaseolorum* VAR. *caulivora*

Organism	Isolate	Percentage reduction in yield in plants inoculated at age of:			
		62 days	76 days	85 days	91 days
D. phaseolorum var. *caulivora*	6	100.0	42.3	23.6	17.6
" " "	10	94.1	34.8	19.8	8.3

Varietal Susceptibility to Stem Canker

No varieties of soybeans presently grown in Ontario are resistant to stem canker. The variety Harman,* however, almost invariably shows lower incidence of the disease than other commercial varieties, with the possible exception of Harosoy.* Technically, Harman and Harosoy are no more resistant to the disease than the apparently much more susceptible varieties, Lincoln, Hawkeye, and Blackhawk. This fact has been clearly demonstrated experimentally. On August 4, 1954, for example, 71-day-old plants of each of the five varieties just mentioned, growing in neighboring rows in the laboratory plots, were inoculated by the toothpick method with Ontario isolate 6 of *D. phaseolorum* var. *caulivora*, and with the Indiana isolate of the same organism obtained from Dr. Athow. In each row, 75 plants were inoculated with each of the two isolates. Within three weeks, all 750 of the inoculated plants were dead except a few whose survival could be ascribed to faulty

*Harman and Harosoy are Canadian varieties developed by C. Owen, Dominion Experimental Station, Harrow, Ontario.

technique. Thus, the "resistance" of Harman and Harosoy is apparent rather than real. No explanation can be offered at the moment why these two varieties remain relatively freer from stem canker than the others mentioned.

In the United States, as in Ontario, differences in the amount of natural infection have been observed among varieties and the extreme susceptibility of Hawkeye was recorded by Weiss (20) in 1952. In tests in 1953 in Indiana, Athow (1) found that Harosoy showed only 5% infection (3% mortality) as compared with 49% (33% mortality) in Hawkeye. In general, however, in the United States, extensive search has failed to find a source of complete resistance to stem canker (12).

Alternate Host Trials

Certain weeds would, if left undistrubed, almost invariably infest soybean fields in southwestern Ontario. They include velvet leaf, *Abutilon theophrasti* Medic., lamb's quarters, *Chenopodium* sp., redroot pigweed, *Amaranthus retroflexus* L., common purslane, *Portulaca oleracea* L., common ragweed, *Ambrosia artemisiifolia* L., and lady's-thumb, *Polygonum persicaria* L. It was thought that one or more of these weeds might serve as an alternate host of the stem canker organism. To investigate this possibility, 25 of each of these weeds, growing either in the laboratory plots or in nearby commercial stands of soybeans, were inoculated during the summer of 1954, by the toothpick method, with isolate 6 of *D. phaseolorum* var. *caulivora*. The weeds were inoculated when they were considered to have reached a point about midway in their seasonal development. None of the inoculated plants showed any sign of infection.

Artificial inoculation of tomato, pepper, and snap and field beans likewise has failed to result in infection.

Identification of the Pod and Stem Blight and Stem Canker Organisms

In a recent publication (11) the present author dealt in some detail with the morphology and sexuality of the fungi associated with pod and stem blight and stem canker of soybeans in Ontario. In that publication, *D. phaseolorum* var. *sojae* was recognized as the cause of pod and stem blight, and the pathogen causing stem canker was referred to as *D. phaseolorum* var. *batatatis*. While the paper in question was in press, Athow and Caldwell (2) published their studies of *Diaporthe* stem canker and pod and stem blight of soybeans in Indiana. In those studies they compared two isolates of *Diaporthe* from sweet potato, presumably *D. phaseolorum* var. *batatatis*, with the pathogen causing stem canker in Indiana. Having found that the two organisms differed pathologically and morphologically, they decided that the name *D. phaseolorum* var. *batatatis* was no longer tenable for the stem canker organism and they proposed that the latter be recognized as a variety of *D. phaseolorum* under the trinomial *D. phaseolorum* var. *caulivora*. In the present studies it has been shown that an Indiana isolate of the stem canker organism is as highly

pathogenic both to seedling and to 71-day-old soybean plants as Ontario isolates of the fungus. Within recent months, the Indiana isolate has been compared in culture with Ontario isolates, and morphologically, they have been found to be indistinguishable from one another, except that certain of the Ontario isolates produce an imperfect stage. It seems to the writer that at the moment too much emphasis should not be placed on the presence or absence of an imperfect stage as a diagnostic characteristic of the stem canker fungus. As imperfect stage may yet be found for the pathogen as it occurs in Indiana. In some of the Ontario isolates the imperfect stage occurred so sparsely that it could have been easily overlooked if the cultures had not been examined most minutely. Reporting recently from Minnesota, Frosheiser and Kernkamp (6) pointed out that in a year's study of the stem canker organism in numerous cultures on potato dextrose agar or on naturally or artificially infested soybean stems in moist chambers no pycnidial formation was observed, but that when stems of certain crop plants and weeds were autoclaved and inoculated with ascospores of the fungus, pycnidia with conidia were observed. Thus, it is not only in Ontario that an imperfect stage of the stem canker organism has been observed.

Because in all respects, except the one of probable minor importance discussed above, the Ontario pathogen agrees so exactly with the organism causing stem canker in Indiana, as described by Athow and Caldwell (2), there is little doubt that they are identical. The present writer adopts, therefore, *D. phaseolorum* var. *caulivora* as the name of the stem canker pathogen in Ontario, and in so doing concurs with Athow and Caldwell (2) in regarding it as a variety of *D. phaseolorum* differing from *batatatis*.

Control

No varieties highly resistant to stem canker and pod and stem blight are known. However, the varieties Harman and Harosoy of Canadian origin have consistently shown a lower incidence of stem canker in Ontario than certain of the more commonly grown introduced varieties. In regions to which they are adapted, then, Harman and Harosoy should be grown in preference to such highly susceptible varieties as Hawkeye and Blackhawk.

The fungi causing pod and stem blight and stem canker overwinter on diseased stems left in the field. The spores produced on such residue serve as a source of inoculum the following spring. Care should be taken, then, to plow under completely the crop residues and to practice crop rotation.

Discussion

In the present investigation certain of the findings have emerged with clarity and certainty and are in full agreement with those of workers elsewhere; others are at variance with previously published reports of work along closely similar lines. Certain of what seem to be the more important of these similarities and dissimilarities are discussed below.

In Iowa, Welch and Gilman (21) readily found, on overwintered soybean stems, the single and cespitose perithecial types representative of *D. phaseolorum* var. *sojae* and *D. phaseolorum* var. *caulivora* (*batatatis*), respectively. In Indiana, Athow and Caldwell (2) reported that the perithecial stage of *D. phaseolorum* var. *sojae* was found much less frequently on overwintered stems than that of *D. phaseolorum* var. *caulivora*. In Ontario, after years of unremitting search, the writer has found the perithecia of *D. phaseolorum* var. *sojae* on only five of several thousand stems that have been critically examined. In Ontario, however, the writer on several occasions has found *before the end end of the growing season* the perithecia of *D. phaseolorum* var. *caulivora* on lesions near the base of the stem of infected plants. Such an observation does not appear to have been recorded previously.

In September 1954, the writer (11) reported the discovery of an imperfect stage of *D. phaseolorum* var. *caulivora*. Almost simultaneously Frosheiser and Kernkamp (6) reported from Minnesota the production of asexual spores by the stem canker fungus. Earlier in 1954, Athow and Caldwell (2) had stated that "the absence of a conidial or imperfect stage offers a distinct morphological basis for separating the stem canker organism from *D. phaseolorum*, *D. phaseolorum* var. *sojae* and *D. phaseolorum* var. *batatatis*". Earlier in the present paper, the writer has expressed the opinion that an imperfect stage may yet be found for the stem canker pathogen in Iowa, and that at the present time too much significance should not perhaps be placed on the presence or absence of an imperfect stage in the stem canker fungus from the diagnostic standpoint.

In 1948, Welch and Gilman (21) paired pycnidial cultures all derived from single ascospores of *D. phaseolorum* var. *sojae*, and in obtaining production of perithecia in certain of the pairings, were successful in demonstrating the heterothallic nature of the Iowa pod and stem blight organism. More recently, Athow and Caldwell (2) in Indiana and the writer (11) in Ontario have failed in repeated similar attempts.

When, earlier in their studies, Athow and Caldwell (2) inoculated plants of different age in the greenhouse with isolates of *D. phaseolorum* var. *caulivora*, they were led to conclude that "there was no appreciable difference in pathogenicity among isolates but the data suggest that the older plants were the more susceptible." The results of later inoculation trials carried out in the field and greenhouse apparently did not cause Athow and Caldwell to modify their earlier conclusion. If that is the case, the findings of the writer are not in accord with those of the investigators in question, because the present investigations have shown rather conclusively that soybean plants of certain varieties at least, instead of becoming more susceptible as they grow older become less so. Moreover, on the basis of physiological specialization some evidence has also been adduced that strains exist among isolates of the stem canker organism.

In describing the symptoms of pod and stem blight Athow and Caldwell (2) state that the disease first appears on the petioles of lower leaves and upon

broken lower branches. They make no mention of having made similar observations with regard to stem canker. As recorded above, the writer has found early in August an abundance of petioles and smaller spurs on the lower part of the plant infected with stem canker and pod and stem blight. How these organs become infected and how significant they may be as a source of inoculum for later infection of plants are questions that invite consideration. If the fungi concerned are indigenous to the soil or are capable of living in it on soybean debris for relatively long periods of time, as Gerdemann (7) has recently suggested with regard to *D. phaseolorum* var. *sojae*, it would seem quite possible that inoculum on soil particles could readily be deposited on petioles and spurs, as the result of splashing. Stems of plants encrusted with soil particles to a height of a foot or more may often be observed. In the present studies no evidence has been found which indicates that inoculum might be present in seedlings as the result of systemic infection. Particularly with regard to *D. phaseolorum* var. *sojae*, the question might arise as to how that organism, which seems to be primarily a facultative parasite, can infect spurs and petioles. By midseason, the upper foliage of normally developing soybean plants has usually filled in between the rows, and cut off the light from the lower parts of the plants. Unable to function normally, lower leaves and spurs, particularly the former, turn yellow and, presumably, their tissues become more or less moribund. In that condition they would seem to be more readily amenable to infection by a facultative parasite like *D. phaseolorum* var. *sojae*. It is true that many of the leaves fall off, first the lamina and then the petiole, but in the aggregate relatively large numbers of the latter remain attached to the stem. Fruit bodies of *D. phaseolorum* var. *sojae* (pycnidia) and of *D. phaseolorum* var. *caulivora* (perithecia), containing mature spores, were found on diseased petioles and spurs. Thus, at midseason, there was present on these organs inoculum which could be carried possibly by insects, by splashing, or by some other means to other parts of the plant such as, for example, leaf scars through which infection so often takes place. Direct spread of stem canker from infected spurs and petioles into the main stem, and subsequent development of a lesion on the latter, was frequently observed. Stems may similarly have been directly infected by the pod and stem blight fungus but infections at the time would have been difficult to detect because under natural conditions and earlier in the season pod and stem blight produces no definite lesion such as is caused by stem canker.

References

1. ATHOW, K. L. Some midwest diseases. Soybean Dig. 14(8) : 19. 1954.
2. ATHOW, K. L. and CALDWELL, R. M. A comparative study of *Diaporthe* stem canker and pod and stem blight of soybeans. Phytopathology, 44 : 319-325. 1954.
3. CHAMBERLAIN, D. W. Soybean disease investigations in 1951. *In* Results of the Cooperative Uniform Soybean Tests, 1951. Part I—North Central States. U.S. Regional Soybean Laboratory, Urbana, Ill. March, 1952.
4. CRALL, J. M. Soybean diseases in Iowa in 1949. Plant Disease Reptr. 34 : 96-97. 1950.
5. CRALL, J. M. A toothpick method of inoculation. (Abstr.) Phytopathology, 42 : 5. 1952.

6. FROSHEISER, F. I. and KERNKAMP, M. F. Asexual spore production in *Diaporthe phaseolorum* var. *batatatis*. (Abstr.) Phytopathology, 44 : 489. 1954.
7. GERDEMANN, J. W. The association of *Diaporthe phaseolorum* var. *sojae* with root and basal stem rot of soybean. Plant Disease Reptr. 38 : 742-743. 1954.
8. HARTER, L. L. Pod blight of the lima bean caused by *Diaporthe phaseolorum*. J. Agr. Research, 11 : 473-504. 1917.
9. HARTER, L. L. and FIELD, E. C. *Diaporthe*, the ascogenous form of sweet potato dry rot. Phytopathology, 2 : 121-124. 1912.
10. HILDEBRAND, A. A. An elaboration of the toothpick method of inoculating plants. Can. J. Agr. Sci. 33 : 506-507. 1953.
11. HILDEBRAND, A. A. Observations on the occurrence of the stem canker and pod and stem blight fungi on mature stems of soybeans. Plant Disease Reptr. 38 : 640-646. 1954.
12. JOHNSON, H. W. Soybean production research. Soybean Dig. 15(11) : 80-82. 1955.
13. KILPATRICK, R. A. and HARTIG, E. E. Effect of planting date on incidence of fungus infection of Ogden soybean seeds grown at Walnut Hill, Fla. Plant Disease Reptr. 39 : 174-176. 1955.
14. LEHMAN, S. G. Pod and stem blight of the soybean. J. Elisha Mitchell Sci. Soc. 38 : 13. 1922.
15. LEHMAN, S. G. Pod and stem blight of soybean. Ann. Missouri Botan. Garden, 10 : 111-178. 1923.
16. LUTTRELL, E. S. *Diaporthe phaseolorum* var. *sojae* on crop plants. Phytopathology, 37 : 445-465. 1947.
17. SASAKI, S. A Phomopsis disease of soybean. Ann. Agr. Expt. Sta. Gov.-Gen. Chosen, 4 : 1-28. 1929. (Abstr. *in* Rev. Appl. Mycol. 9 : 83. 1930).
18. TUCKER, C. M. *Diaporthe phaseolorum* on pepper fruits. Mycologia, 27 : 580-585. 1935.
19. WEHMEYER, L. E. The genus *Diaporthe* and its segregates. University of Michigan Press, Ann Arbor, Mich. 1933.
20. WEISS, M. G. Agronomic research goals in soybean. Soybean Dig. 12 : 57. 1952.
21. WELCH, A. W. and GILMAN, J. C. Hetero- and homo-thallic types of *Diaporthe* on soybeans. Phytopathology, 38 : 628-637. 1948.
22. WELLS, D. G., HARE, W. W., and WALKER, J. C. Evaluation of resistance and susceptibility in garden pea to near-wilt in the greenhouse. Phytopathology, 39 : 771-779. 1949.
23. WOLF, F. A. and LEHMAN, S. G. Notes on new or little known plant diseases in North Carolina in 1920. North Carolina Agr. Expt. Sta. Ann. Rept. 43 : 55-58. 1920.
24. WOLF, F. A. and LEHMAN, S. G. Diseases of soybean which occur both in North Carolina and the Orient. J. Agr. Research, 38 : 381-386. 1926.

THE PHYSIOLOGY OF HOST–PARASITE RELATIONS

II. THE EFFECT OF PUCCINIA GRAMINIS TRITICI ERIKS. AND HENN. ON THE RESPIRATION OF THE FIRST LEAF OF RESISTANT AND SUSCEPTIBLE SPECIES OF WHEAT[1]

By D. J. Samborski and Michael Shaw

Abstract

The first leaves of resistant (var. Khapli) and susceptible (var. Little Club) species of wheat were heavily inoculated with *P. graminis* (Race 15B). The effect of the frequency of the pustules on their size and on the leaves is described. The oxygen consumption, total dry weight, total nitrogen, and sugar contents of leaf disks were measured at frequent intervals after inoculation. At infections of Little Club massive increases in dry weight occurred in 10 to 15 days, but the percentage total nitrogen remained close to that of healthy leaves. Oxygen consumption per unit dry weight or per unit of tissue nitrogen rose to from 2.5 to 3.0 times that for healthy tissue and then fell off again. The duration and degree of the rise were dependent on environmental conditions. At infections on Khapli, both dry weight and the percentage total nitrogen declined rapidly and the leaves turned yellow and died in about eight days, suggesting a rapid breakdown of protein. Oxygen consumption per unit dry weight or per unit of tissue nitrogen rose more rapidly, to from 2.0 to 2.5 times that of healthy tissue, and declined again sooner than at infections on Little Club. With both species, the 'accumulation' at infections of radioactive glucose, or its products, roughly paralleled the course of respiration. With leaves of Little Club, initially low in carbohydrate and having a low respiratory quotient, a marked rise in the respiratory quotient occurred at developing infections; little or no rise occurred with Khapli. The results of the sugar analyses suggested that the increase in respiration following infection was not initially dependent on an increase in the sugar content above that found in the healthy leaf. The significance of the results is discussed.

Introduction

In Part I (15) it was shown that the increase in respiration caused by the obligate parasites, *Puccinia* and *Erysiphe*, is accompanied by an accumulation or mobilization, at the infections, of various substances including carbon compounds. Accumulation was shown to be dependent on aerobic respiration; it was also pointed out that marked local increases in dry weight, and sometimes also an actual hypertrophy of the host cells, have been reported at uredial infections of rust (19, 20). On the basis of extended observations Sempio (14) has formulated a metabolic theory of disease resistance in which he emphasizes the importance of the relative rates of photosynthesis, respiration, and fermentation. Consideration of the results of genetic studies, such as those of Flor (5–8), also suggests that resistance and susceptibility depend in a very critical manner on the interlocking of metabolic systems or the mutual adjustment of metabolic rates. Apart from the work of Gassner and his associates (9) on nitrogen content, quantitative data have not been available for the comparison of the physiological effects of a single pathogen on resistant and susceptible hosts. In the belief that such comparative data

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Contribution from the Plant Physiology Laboratory, Department of Biology, University of Saskatchewan, Saskatoon, Sask.

are essential for the understanding of host–parasite relations and the nature of resistance, the work reported in this paper was carried out with the primary object of determining the rate of change of respiration, after inoculation with stem rust, of the first leaves of resistant and susceptible species of wheat.

Materials and Methods and Preliminary Observations

Host–Parasite Combinations

The host–parasite combinations employed are shown in Table I. Ideally, it would be desirable to investigate the effect of a single physiological race of the rust fungus on resistant and susceptible varieties of wheat which were otherwise as similar genetically as possible ('isogenic'). In fact, in the present work two species—Khapli and Little Club—have been used. This choice of hosts was dictated largely by practical considerations, the chief of which was the relative constancy and difference between their reactions to the rust under

TABLE I

Organism	Host	Reaction type
Puccinia graminis tritici Eriks. and Henn. (Race 15B)	*Triticum compactum* Host (var. Little Club)	4
	T. dicoccum Schübler (var. Khapli)	1
	'Khapli I'. An 'off-type' maintained in the Dept. of Biology plots	2-3⁻
Erysiphe graminis hordei El. Marchal (Race 3)	*Hordeum vulgare* L. (var. Atlas)	4
	H. vulgare L. (var. Atlas 46)	Brown necrotic flecks. No macroscopically visible mycelium

greenhouse conditions. The importance of genetic control of the host is well illustrated by the differences, described later, between the rust reactions of Khapli and Khapli I (see also Table I). The varieties of barley, Atlas and Atlas 46, used as hosts for *Erysiphe*, while not 'isogenic', are nearly so (12). The wheat or barley seed was treated with a mixture of ceresan and talc (1 : 5) to eliminate seed-borne pathogens and was planted in pots or flats. Four to five grams of ammonium phosphate fertilizer (11-48-0) was added to each cubic foot of soil (one part sand, three parts loam). Race 15B of *Puccinia* was 'cultured' continuously on successive 'crops' of Atlas 46 barley in the greenhouse. The uredospores were harvested and stored in the refrigerator. Race 3 of *Erysiphe* was maintained on Atlas barley.

Inoculation

The first seedling leaves, which were used throughout this investigation, were inoculated with rust (Race 15B, except where specified) about 10 days

after planting, when the second leaf was emerging. A suspension of uredospores in water, together with a little gelatin and 100 p.p.m. of aureomycin, was rubbed onto each leaf by hand. While the leaves were still moist, the plants were placed in a humid atmosphere in darkness for 18 to 24 hr. Control plants were similarly treated after the leaves had been rubbed with a solution of gelatin and aureomycin. The barley plants were also inoculated, by heavy dusting with mildew conidia, at approximately 10 days after planting.

Sampling was usually started before the efficiency of the inoculation could be determined. However, the results from such samples were accepted only when a heavy infection developed over the entire leaf surface.

The notes in Table II summarize the effect of the heaviness of rust infection on the leaves of Little Club and Khapli, while the data in Table III illustrate that there is an inverse linear relationship between frequency and size of pustule, as measured by the area of the sporulating surface. Areas were measured by planimetry. The infection frequency clearly plays an important role in determining the degree of development at each infection as well as the reaction of the leaf as a whole, particularly with Khapli.

TABLE II

THE EFFECT OF HEAVINESS OF INFECTION WITH RUST (RACE 15B) ON THE FIRST SEEDLING LEAVES OF LITTLE CLUB AND KHAPLI WHEATS

Infection	Little Club	Khapli
Light	1–20 large pustules per 3 cm. length of leaf. Very heavy sporulation. Secondary uredinia form. No chlorosis	Up to 40 small sporulating centers surrounded by white necrotic areas per 3 cm. length of leaf. No chlorosis outside infection site
Heavy	50–100 small sporulating pustules per 3 cm. length of leaf. Secondary sporulation delayed or absent. No chlorosis for 10–12 days. Death may result from drying out when leaves still green	Entire leaf yellows in 4–5 days. 75–100 infection-centers, visible as minute green areas on yellow background, per 3 cm. length of leaf. No sporulation and no evidence of local necrosis. Death results from drying out from tip in 8 days

TABLE III

THE RELATION BETWEEN FREQUENCY AND SIZE OF PUSTULE, AS MEASURED BY AREA OF SPORULATING SURFACE, ON RUST-INFECTED LEAVES OF LITTLE CLUB WHEAT

Pustules/3 cm.* (F)	$1/F$	Total pustule area per 3 cm. (cm.^{2})	Area(A)/pustule (cm.^{2})
1	1	0.1728	0.1728
4	0.250	0.2217	0.0554
13	0.077	0.2943	0.0226
20	0.050	0.2217	0.0111
52	0.019	0.2686	0.0052

**A section 3 cm. long was taken from the center of each leaf about 12 days after inoculation. Mean area per 3 cm. = 1.62 cm.^{2} $1/F = 5.9245A - 0.0384$; $r = +0.997$; $P < 0.01$.*

Respiratory Measurements

Respiration rates and respiratory quotients were determined with the Warburg apparatus at 25° ± 0.02° C. in darkness as described in Part I (15). The first leaves were cut into disks (diameter 2.8 or 5 mm.) or pieces (length 5 mm.) with a stainless steel cutter or new razor blades and floated on 0.05 *M* phosphate buffer at pH 5.5. Using comparable heavily-infected leaves, the disks or pieces were cut to include infection-centers, since the respiration rate falls off rapidly with increasing distance from the infections. Data which illustrate this point and which were obtained with lightly-infected leaves are presented in Table IV. Respiratory measurements were always started approximately one and one-half hours after the leaves were cut and continued for from one to three hours.

Repeated examinations of the buffer solution at the end of the Warburg experiments gave no indication of bacterial contamination. Most of the 'free' uredospores were knocked off the leaves during the preparation of the disks; of those which remained and were later washed off the tissue into the buffer, none were ever observed to germinate, even during experiments lasting for six hours.

Finally, it should be emphasized that the respiratory rates obtained for infected leaf disks represent the sums of the rates of both fungal and host tissues.

TABLE IV

THE RELATION BETWEEN RESPIRATION RATE AND DISTANCE FROM THE INFECTION-CENTER OF DISKS CUT FROM RUST-INFECTED LITTLE CLUB LEAVES

	Distance of disk center from pustule center (mm.)	Dry wt. of 60 2.8-mm. disks (mgm.)	Resp./60 disks (μl. O_2/hr.)	Resp. (μl./10 mgm. dry wt./hr.)
Infected leaves	0	14.55	70.0	48.5
	4	6.00	15.3	25.4
	8	4.75	10.0*	21.0*
	12	4.40	9.1*	20.7*
Uninfected leaves	—	5.30	9.7*	18.3*

**Differences not significant* ($P > 0.05$).

Dry Weights

Dry weights were obtained after quantitative recovery of the plant tissue from each flask at the completion of each Warburg experiment. Drying was carried out in a forced-air oven at 95° C. for 12 to 18 hr.

Autoradiography

The leaves were fed with glucose-1-C^{14} and treated and radiographed as described in Part I (15).

Nitrogen Determinations

These were made in duplicate by a 'semimicro' modification of the procedures given in (3).

Carbohydrate Determinations

The procedures outlined by Wylam (18) for carbohydrate extraction and "clearing" were closely followed. The first leaves were excised, capped in tared weighing bottles, and weighed (subsamples were taken at the same time for determination of dry weight). After weighing, the leaves were dropped into boiling 90% ethanol (approx. 35 ml. for 1 gm. fresh weight of leaves) and refluxed for 30 min. The extraction was repeated with a second volume of ethanol and the extracts combined. Preliminary chromatography had demonstrated the absence of free xylose in the wheat leaves. A known amount of xylose was therefore added to the leaf material and ethanol. This xylose, quantitatively estimated later in the analysis, indicated the fraction of the total extract represented by that portion spotted on the chromatography sheet.

The ethanol was removed *in vacuo* at 40° C. and the aqueous extract was "cleared" by addition of equal volumes of barium hydroxide and cadmium sulphate of equivalent normalities, followed by passage through amberlite IRI20 and IR4B resins.

The solution was concentrated and the carbohydrates separated by paper chromatography (water: butanol: acetic acid, 5 : 4 : 1). Carbohydrates were located on guide strips with a benzidine reagent (17). Appropriate areas of the chromatogram were then cut up and eluted overnight at 6° C. in 10 ml. of water. The eluate was stirred with a glass rod and centrifuged, and an aliquot removed for colorimetric development. Sucrose, glucose, and fructose were determined using an anthrone method (13). The method of Somogyi (11) was used for xylose.

Experimental Results

Fig. 1 shows that, per unit area, the oxygen consumption at rust infections reached a maximum not later than the sixth day after inoculation for Khapli (light infection, type 1^{-}-1 pustules; see Table II), on the eighth day for Khapli I (infection type 2-3^{-}), and not before the 12th day for Little Club (infection type 4). The rate of increase in respiration was approximately the same in all three cases. However, the respiratory peak was reached soonest on the most resistant and latest on the most susceptible of the three wheats. The main events in the development of these infections are given in Table V. Fig. 3 shows that the accumulation of radioactive glucose (determined at the same time that the respiratory measurements were made) was pronounced at the type 4 infections on Little Club by the sixth day, and increased at least up to the 10th day after inoculation. Fig. 4 shows that accumulation increased up to the eighth day at the type 2-3^{-} infections on Khapli I, but did not occur on the 10th day, on which, as Fig. 1 shows, the rate of oxygen consumption had fallen sharply to approximately 60% of the maximal value observed on the eighth day. The drop in respiration (Fig. 1) and failure in accumulation were both associated with an increase in necrosis at the infections (Table V). Radiographs of infected Khapli leaves showed a similar parallelism between accumulation at the infections and the respiration rate.

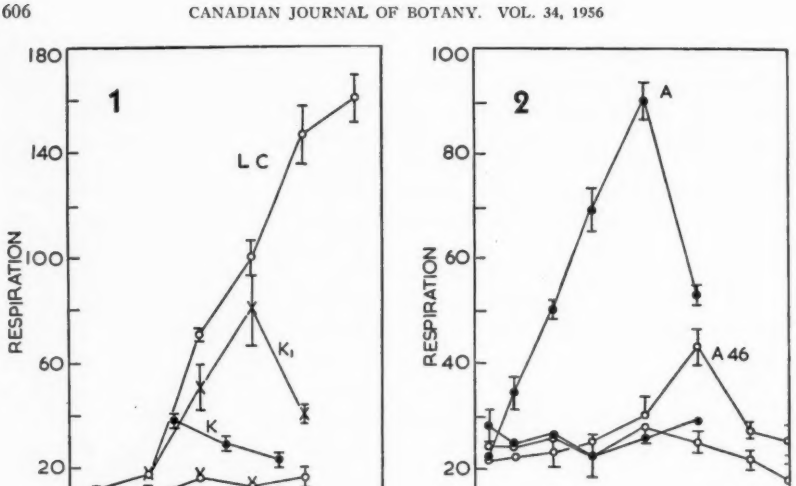


FIG. 1. The effect of stem rust (mixed inoculum: Races 15B and 56) on the respiration of the first leaves of Little Club (LC), Khapli I (KI), and Khapli (K) wheats. The unlabelled curves give the respiration for uninfected leaves of each 'variety'. Each point is the mean of three to five determinations. Vertical lines in this and subsequent figures are twice the standard deviations (s). Values of s less than twice the width of the symbols used are not shown. Ordinate: microliters of oxygen consumed per 35 leaf-disks (diam. 5 mm.) per hour. Abscissa: days after inoculation.

FIG. 2. The effect of mildew (Race 3) on the respiration of the first leaves of Atlas (A) and Atlas 46 (A46) barleys. Conventions, etc. as for Fig. 1.

TABLE V

MAIN EVENTS IN THE DEVELOPMENT OF THE RUST INFECTIONS FOR WHICH THE RESPIRATORY DRIFTS ARE ILLUSTRATED IN FIG. 1

Date 1954	Days after inoculation	Little Club	Khapli I	Khapli
Apr. 1	—	Planted	Planted	
Apr. 11	0	Inoculated	Inoculated	
Apr. 15	4	—	Flecks visible	
Apr. 16	5	Flecks visible	—	
Apr. 17	6	Sporulation		
Apr. 19	8	Vigorous sporulation. Type 4	Sporulation. Type 2-3⁻. Yellowing beginning	
Apr. 21	10	—	Top halves of leaves yellow. Increased necrosis at infections	
Apr. 23	12	Leaf tips yellowing*	Leaves nearly all dead	
June 7	—			Planted
June 15	—			Inoculated
June 18	3			Flecks visible
June 20	5			Moderate infection. Flecks white. Traces of sporulation
June 22	7			White, necrotic pustules. Type 1⁻-1
June 24	9			Increased necrosis at infections. Leaves yellowing

NOTE: *In these experiments the inoculum was a mixture of uredospores of Races 56 and 15B.*
**Rusted leaves, dry weight = 23.9% fresh weight. Healthy leaves dry weight = 11.1%.*

Iodine tests showed that the deposition of starch around infections on Little Club roughly paralleled respiration and the accumulation of radioactive glucose, reaching a maximum on the 8th to 10th days after inoculation and declining thereafter. Less starch was found in association with the infections on Khapli I and none at all at infections on Khapli in this or in any other experiment. Under conditions of reduced illumination, no starch formation occurred even at infections on Little Club.

Although the experiment with Khapli was carried out two months later than those with the other two varieties, data from other experiments have confirmed the general relationship between the three curves in Fig. 1.

For comparison with the foregoing, the curves in Fig. 2 and the notes in Table VI illustrate the effect of Race 3 of *E. graminis* on two varieties of barley, Atlas and Atlas 46. As Fig. 2 shows, no distinct respiratory increase occurred on Atlas 46 until after the respiratory maximum was reached on Atlas. The delayed and transitory respiratory rise on Atlas 46 was associated with the formation of brown necrotic flecks. On Atlas, accumulation of radioactive glucose paralleled the development of the mildew colonies (15). Accumulation was not detected, macroscopically, at infections on Atlas 46, even though a number of haustoria were produced.

TABLE VI

MAIN EVENTS IN THE DEVELOPMENT OF THE MILDEW INFECTIONS FOR WHICH THE RESPIRATORY DRIFTS ARE ILLUSTRATED IN FIG. 2

Date 1954	Day after inoculation	Atlas	Atlas 46
Mar. 3	—	Planted	Planted
Mar. 14	0	Inoculated	Inoculated
Mar. 16	2	Halos at penetration points. Haustoria	Halos at penetration points. Haustoria
Mar. 17	3	Flecks visible	Flecks visible
Mar. 19	5	Small colonies	A few necrotic flecks
Mar. 22	8	Large, vigorous colonies	—
Mar. 29	15	Cleistothecial formation	—
Apr. 2	19	Leaf tips drying. Green islands on yellowing leaves	Numerous small necrotic flecks. Leaves yellowing, especially at tips
Apr. 6	23	All first leaves dead	—
Apr. 9	26	—	Many first leaves completely yellow. Flecks dark brown

In Tables VII to X inclusive, data are presented to show the changes in dry weight, total nitrogen, and oxygen consumption during the progress of development of Race 15B of *Puccinia* on Khapli and Little Club wheats. Figures for the mean daily hours of direct sunshine during each experiment are also given (Tables VII and IX). These were obtained from the records of the Physics Department and serve as a rough indication of the level of illumination in the greenhouse. On Little Club, infection was followed by

slight initial losses in dry weight during the first one to four days after inoculation, but later by massive increases of up to 100% (Fig. 5) to 200%. In general the rate of increase in dry weight was dependent on environmental conditions, being most rapid under continuous illumination (Expt. VIII.L., Table VII, and Fig. 5). With lightly-infected Khapli leaves (type 1 pustules, see Table II) the dry weight remained close to that of healthy tissue (Expt. I.K., Table IX, Fig. 5). On the other hand, on heavily-infected leaves of Khapli (no sporulation, see Table II) there was no recovery from an initial loss in dry weight, which reached 25% in six or seven days in Experiments II*a*.K. and V.K. (Table IX, Fig. 5).

TABLE VII

THE EFFECT OF RUST INFECTION ON THE DRY WEIGHT AND NITROGEN CONTENT OF THE FIRST SEEDLING LEAF OF LITTLE CLUB WHEAT

Expt.	Date 1955	Days after inoculation	Mean daily sunshine* (hr.)	Dry wt. per 60 2.8 mm. disks (mgm.) H	Dry wt. per 60 2.8 mm. disks (mgm.) R	Total N (% dry wt.) H	Total N (% dry wt.) R
I.L.	Feb. 4	3		7.18	7.06	—	—
	Feb. 7	6	2.42	5.78	6.78	5.60	5.36
	Feb. 10	9		7.55	9.00	5.14	5.07
II.L.	Feb. 22	14	5.04	6.53	10.80	4.72	4.35
	Feb. 25	17		7.00	14.45	5.32	4.97
III.L.	Mar. 3	3	4.76	9.03	8.70	6.09†	6.07
	Mar. 8	8		8.23	11.48	5.63	5.48
IV.L.	Apr. 25	3		9.03	7.73	6.31	6.30
	Apr. 27	5		8.15	7.71	—	5.29
	Apr. 29	7	5.53	7.60	8.53	—	4.83
	May 2	10		6.93	8.56	4.75†	4.83
V.L.	May 3	13	5.12	11.03	15.66	5.00	4.95
	May 4	14		9.40	13.64	4.35	4.64
VI.L.	May 25	13	7.54	7.80	10.20	4.29	4.77
VII.L.	July 19	8		—	—	—	—
	July 20	9	11.06	—	—	—	—
	July 22	11		—	—	—	—
	July 26	15		—	—	—	—
VIII.L.	Nov. 18	4		—	—	6.00	5.90
	Nov. 21	7	2.82	8.95	14.15	—	—
	Nov. 24	10		9.65	17.55	5.27	5.27
	Nov. 29	15		—	—	4.46	4.07

**Hours of direct sunshine per day from the day following inoculation until the end of the experiment. In Experiments I–III low intensity supplementary lighting was provided during the day by incandescent lamps. In Experiment VIII fluorescent lamps provided continuous supplementary lighting at an intensity of 1000 ft-c., approximately. Heavy infections developed in all experiments. Dry weights are means of two to four (for healthy disks) or two to eight (for rusted disks) determinations. Nitrogen values are means of two determinations.*

†Indicates single determinations only.

PLATE I

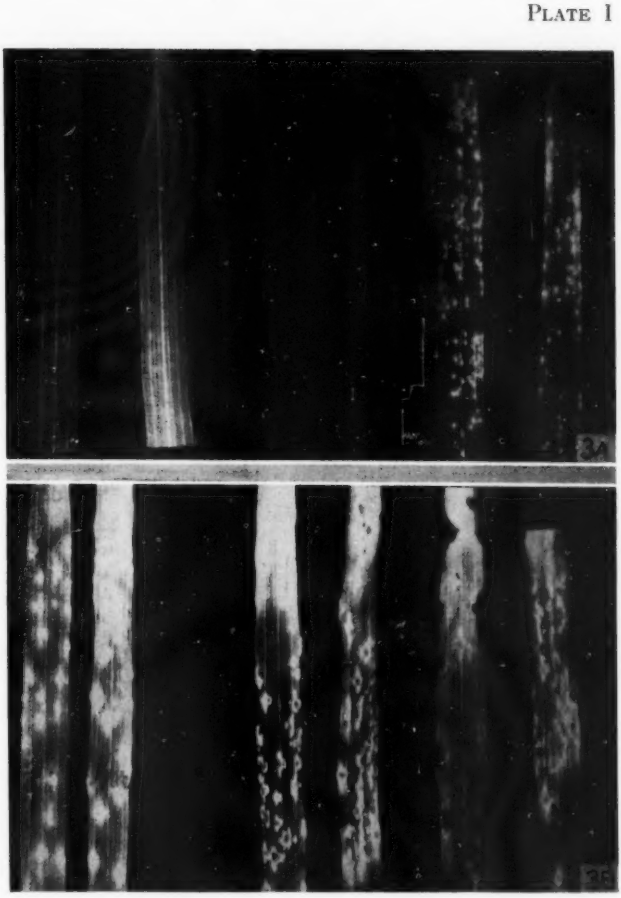


FIG. 3. Radiographs of excised first leaves of Little Club bearing infections of stem rust (mixed inoculum: Races 15B and 56) after feeding with a 0.5% (w/v) solution of glucose-1-C^{14}. The radiographs in both Figs. 3 and 4 were made using samples of the same material for which the respiratory drifts are illustrated in Fig. 1. Fig. 3A: Two, four, and six days after inoculation. Note accumulation of radioactivity at the infections on the sixth day. Fig. 3B: Eight, 10, and 12 days after inoculation. Note decreased accumulation on the 12th day.

PLATE II

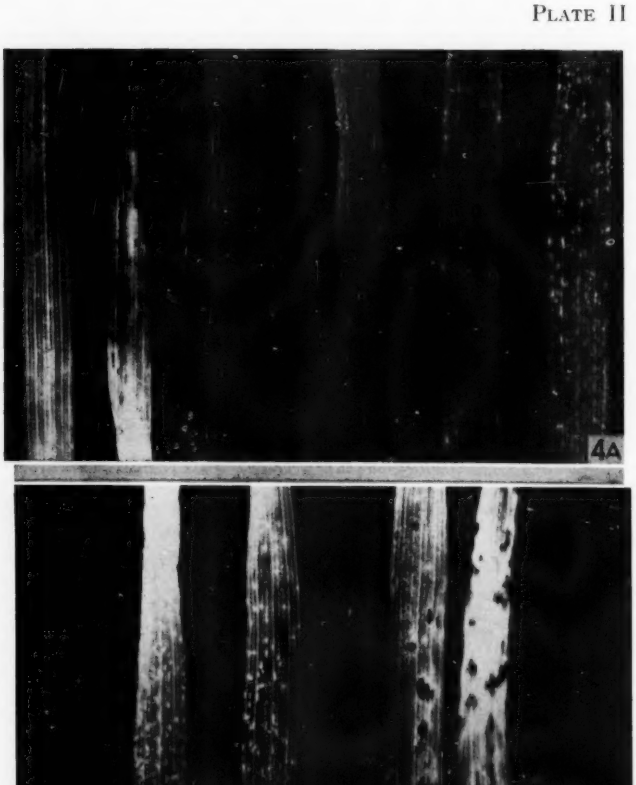


FIG. 4. Radiographs of excised first leaves of Khapli I bearing infections of stem rust (mixed inoculum: Races 15B and 56) after feeding with a 0.5% (w/v) solution of glucose-1-C^{14}. Fig. 4A: Two, four, and six days after inoculation. Note accumulation at infections on sixth day. Fig. 4B: Eight and 10 days after inoculation. Note lack of accumulation, associated with necrosis, on 10th day.

TABLE VIII

THE EFFECT OF RUST INFECTION ON THE OXYGEN CONSUMPTION AND RESPIRATORY QUOTIENT OF THE FIRST SEEDLING LEAF OF LITTLE CLUB WHEAT

Expt.	Date 1955	Days after inoculation	O_2 uptake (μl./hr.) Per 60 2.8 mm. disks H	Per 60 2.8 mm. disks R	Per 10 mgm. dry wt. H	Per 10 mgm. dry wt. R	Per mgm. tissue nitrogen H	Per mgm. tissue nitrogen R	R.Q. H	R.Q. R
I.L.	Feb. 4	3	16.8	17.3	23.4	24.5	—	—	0.727	0.780
	Feb. 7	6	15.1	18.2	26.1	26.8	46.6	50.0	0.674	0.764
	Feb. 10	9	18.7	39.6	24.8	44.0	48.2	86.8	0.758	0.965
II.L.	Feb. 22	14	12.2	52.7	18.7	48.8	39.6	112.2	0.650	0.906
	Feb. 25	17	17.6	72.8	25.1	50.4	47.2	101.4	0.695	0.925
III.L.	Mar. 3	3	17.6	18.8	19.5	21.6	32.0	35.6	0.770	0.745
	Mar. 8	8	17.3	55.5	21.0	48.3	37.3	88.1	0.745	0.963
IV.L.	Apr. 25	3	26.1	21.0	28.9	27.2	45.8	43.2	0.949	0.850
	Apr. 27	5	21.0	28.4	25.8	36.8	—	69.6	0.824	0.834
	Apr. 29	7	21.4	39.7	28.2	46.5	—	96.3	0.965	0.910
	May 2	10	14.7	32.2	21.2	37.6	44.6	77.8	0.756	0.793
V.L.	May 3	13	20.0	73.9	18.1	47.2	36.2	95.4	—	—
	May 4	14	12.7	47.5	13.5	34.8	31.0	75.0	0.844	0.985
VI.L.	May 25	13	16.2	49.8	20.8	48.8	48.5	102.0	—	—
VII.L.	July 19	8	—	—	15.7	36.6	—	—	0.971	0.940
	July 20	9	—	—	14.2	35.3	—	—	0.924	0.952
	July 22	11	—	—	15.6	38.2	—	—	1.010	0.947
	July 26	15	—	—	14.6	35.4	—	—	—	—
VIII.L.	Nov. 18	4	—	—	26.3	34.9	43.8	59.2	—	—
	Nov. 21	7	—	—	21.4	53.5	—	—	—	—
	Nov. 24	10	—	—	24.8	64.0	47.0	121.4	—	—
	Nov. 29	15	—	—	—	74.3	—	182.6	—	—

NOTE: *O_2 uptake values are means of two to eight determinations. Respiratory quotients are means of two to four determinations. In Experiments VII and VIII leaf-pieces approximately 0.5 cm. long were used for measurement of O_2 uptake.*

The data for Experiment I have been plotted on a telescoped time scale in Figs. 7 and 9, because rust development was delayed initially owing to the low level of illumination (see Table VII).

TABLE IX

THE EFFECT OF RUST INFECTION ON THE DRY WEIGHT AND NITROGEN CONTENT OF THE FIRST SEEDLING LEAF OF KHAPLI WHEAT

Expt.	Date 1955	Days after inoculation	Mean daily sunshine (hr.)	Dry wt. per 60 2.8 mm. disks (mgm.) H	Dry wt. per 60 2.8 mm. disks (mgm.) R	Total N (% dry wt.) H	Total N (% dry wt.) R
I.K.	Feb. 11	3		7.18	7.03	—	—
	Feb. 16	8	5.19	6.33	6.65	6.01	4.47
	Feb. 19	11		7.08	7.23	4.61	4.03
IIa.K.	Apr. 4	3		8.83	7.50	5.61	4.85
	Apr. 6	5	6.67	8.98	7.70	5.25*	4.15
	Apr. 8	7		8.20	6.44	5.16*	2.98
IIb.K.	Apr. 25	8	—	—	5.48	—	—
V.K.	Nov. 23	5	1.86	7.75	5.05	7.13	4.39
	Nov. 25	7		8.63	—	6.74	3.73

**Single determinations only.*

Replications as for Table VII. Experiment I—light infection. Experiments II to V—heavy infections. In Experiments III, IV (Table X), and V fluorescent lamps provided continuous supplementary illumination at an intensity of 1000 ft-c. approximately.

TABLE X

THE EFFECT OF RUST INFECTION ON THE OXYGEN CONSUMPTION AND RESPIRATORY QUOTIENT OF THE FIRST SEEDLING LEAF OF KHAPLI WHEAT

Expt.	Date 1955	Days after inoc.	O_2 uptake (μl./hr.) Per 60 2.8 mm. disks H	R	Per 10 mgm. dry wt. H	R	Per mgm. tissue nitrogen H	R	R.Q. H	R
I.K.	Feb. 11	3	13.8	15.5	19.2	20.0	—	—	0.765	0.710
	Feb. 16	8	14.8	19.7	23.4	29.6	39.0	66.2	0.785	0.800
	Feb. 19	11	13.3	22.5	18.8	31.1	40.8	77.2	0.770	0.805
II*a*.K.	Apr. 4	3	20.0	27.5	22.7	36.7	40.5	75.7	0.885	0.910
	Apr. 6	5	22.9	35.8	25.5	46.5	48.6	112.0	0.997	0.937
	Apr. 8	7	14.3	19.1	17.4	29.7	33.7	99.7	0.844	0.870
II*b*.K.	Apr. 25	8	—	10.0	—	18.2	—	—	—	—
III.K.	Sept. 28	5	—	—	24.2	49.4	—	—	—	—
IV.K.	Nov. 8	4	—	—	31.6	82.1	—	—	—	—
V.K.	Nov. 23	5	—	—	29.3	62.2	41.1	141.7	—	—
	Nov. 25	7	—	—	27.4	36.5	40.7	97.9	—	—

NOTE: *Replications as for Table VIII. In Experiments III, IV, and V leaf-pieces approximately 0.5 cm. long were used for measurement of O_2 uptake.*
The data for Experiment I have been plotted on a telescoped time scale in Figs. 7 and 9 because rust development was delayed initially and only a light infection developed.

Fig. 6 illustrates the drifts in nitrogen content at infections on the two species. At infections on Little Club the nitrogen content remained close to that of healthy leaves, though there was an indication of a slight initial drop, followed by a rise and a more marked secondary decline. Again, with Khapli, a rapid fall was observed with heavily-infected leaves and the nitrogen content in Experiments II*a*.K. and V.K. fell in five or six days to 50–60% of that of healthy leaves. However, with a light infection there was evidence of a recovery from an initial loss (Expt. I.K., Table IX, Fig. 6). These data

FIG. 5. The drifts in dry weight at rust infections (Race 15B) on the first leaves of Little Club (LC) and Khapli (K). Ordinate: dry weight of 60 leaf-disks (diam. 2.8 mm.) as a percentage of the weight of disks cut from uninfected leaves. Abscissa: days after inoculation. Primary data are given in Tables VII and IX. Symbols: ● Expt. I; × Expt. II; ○ Expt. III; □ Expt. IV; ⊙ Expt. V; △ Expt. VI; ▽ Expt. VII; ⊖ Expt. VIII.

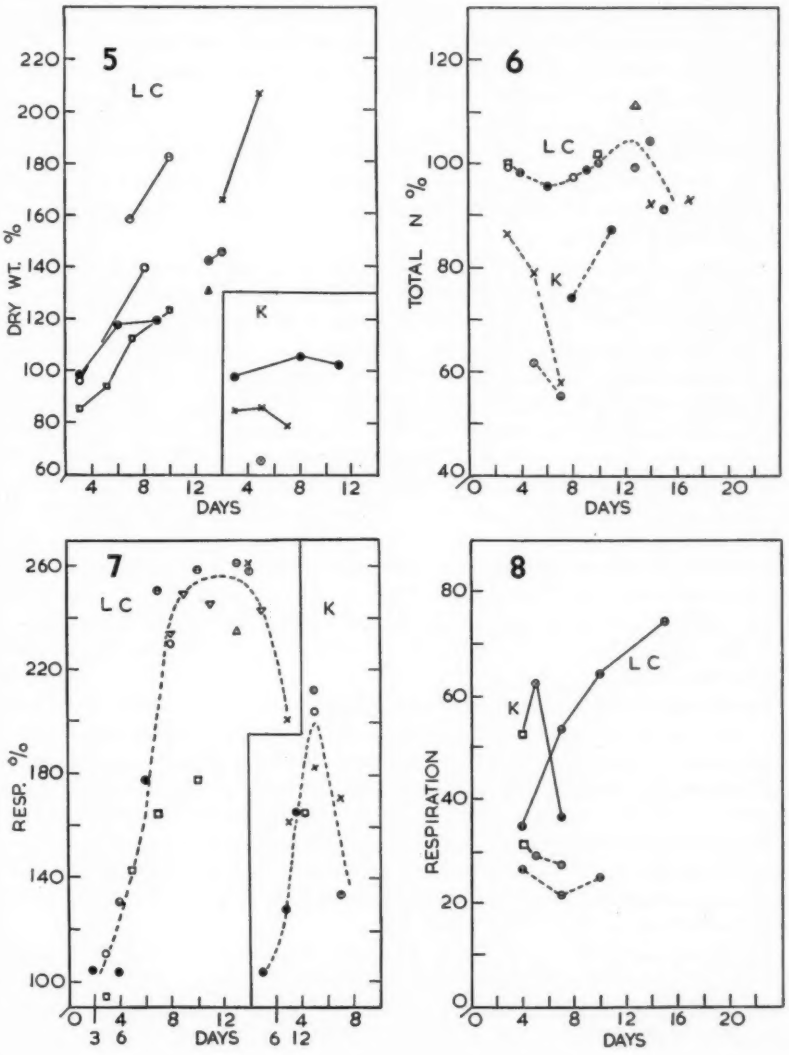
FIG. 6. The drifts in total nitrogen content at rust infections (Race 15B) on the first leaves of Little Club (LC) and Khapli (K). Ordinate: gm. of total N per 100 gm. dry weight as a percentage of the value for uninfected leaves. Abscissa: days after inoculation. Primary data are given in Tables VII and IX. Symbols: as for Fig. 5. In Expt. I.K. infection was light (see text).

FIG. 7. The drifts in respiration at rust infections (Race 15B) on the first leaves of Little Club (LC) and Khapli (K). Ordinate: oxygen consumption per 10 mgm. dry weight per hour as a percentage of the value for uninfected leaves. Abscissa: days after inoculation. Note that the time scales for Expts. I.L. and I.K. have been telescoped. See footnote to Tables VIII and X. Primary data are given in Tables VIII and X. Symbols: as for Fig. 5. The broken lines were drawn arbitrarily.

FIG. 8. The drifts in respiration at rust infections (Race 15B) on the first leaves of Little Club (LC) and Khapli (K). Solid lines, infected leaf-pieces. Broken lines, uninfected leaf-pieces. Ordinate: microliters of oxygen consumed per 10 mgm. dry weight per hour. Abscissa: days after inoculation. Primary data are given in Tables VIII and X. Symbols: as for Fig. 5.

then, though not extensive, delineate the main trends with respect to changes in dry weight and total nitrogen at rust infections on the first leaves of the two species Little Club and Khapli.

Returning now to a consideration of the respiratory drifts, the 'freehand' curves in Fig. 7, plotted from the primary data in Tables VIII and X, illustrate the respiratory drifts per unit dry weight at infections of Race 15B on Little



Club and Khapli. In both species, after an initial lag period, the respiration rose approximately logarithmically and then fell off again. Close comparison of the two curves shows, however, that the lag period was shorter for Khapli, and that the rise in respiration was more rapid, reaching approximately 180% of control values by the fourth day, while the peak value, approximately 200%, was reached on the fifth day after inoculation and was followed by a rapid decline. On the other hand the respiration at infections on Little Club hardly exceeded 140% by the fourth day. The respiratory peak, 50–60% higher than that at infections on Khapli, was not reached until the 10th or 12th day after inoculation and was then followed by a decline, which was often more leisurely than in Khapli.

The more rapid rise and decline in the respiration at infections on Khapli as compared with those on Little Club is more clearly demonstrated by the curves in Fig. 8 plotted from the data for Experiments IV.K., V.K., and VIII.L., carried out at approximately the same time under conditions of continuous supplementary lighting.

The same trends are also evident when the respiration rates are expressed on the basis of total tissue nitrogen (Fig. 9). In infected Khapli the rate of oxygen consumption per unit of tissue nitrogen rose approximately twice as fast as that for infected Little Club. The curves in Fig. 9 also show that the degree and duration of the respiratory rise in infected Little Club leaves were, in general, dependent upon environmental conditions. Thus, the respiratory rise lasted for 15 days in Experiment VIII.L., carried out under continuous supplementary lighting, but only for seven to nine days in Experiment I.L., III.L., and IV.L., during which the natural illumination was low (Table VII). Moreover the respiratory maximum in Experiment VIII.L. was about three times that in the latter experiments. It is noteworthy, however, that the initial rate of increase in respiration was approximately the same in all these experiments with rusted Little Club (Fig. 9). The data for Experiments I.K., II*a*.K., and V.K. with Khapli (Table X) possibly also conform to this pattern, and the appropriate curves in Fig. 9 have been drawn in accordance with this assumption.

Measurements of the respiratory quotient are recorded in Tables VIII and X. The respiratory quotient of healthy leaves showed an orderly progression from about 0.75 to about 0.97 with the improvement in growing conditions in the greenhouse from winter (February and March) to summer (July), as shown by Fig. 10. Under winter, but not under summer conditions, rust infection caused a marked increase in the respiratory quotient of leaf-disks of Little Club but had little effect on the respiratory quotient of Khapli under any conditions (Fig. 11). This result undoubtedly reflects the mobilization of carbohydrate at type 4 infections.

In Experiments IV.L. and II*a*.K. analyses for soluble carbohydrates were carried out and the data obtained are presented in Table XI. Although scant, they present several interesting features. In the first place there was, in agreement with the foregoing, a positive correlation between the respiratory

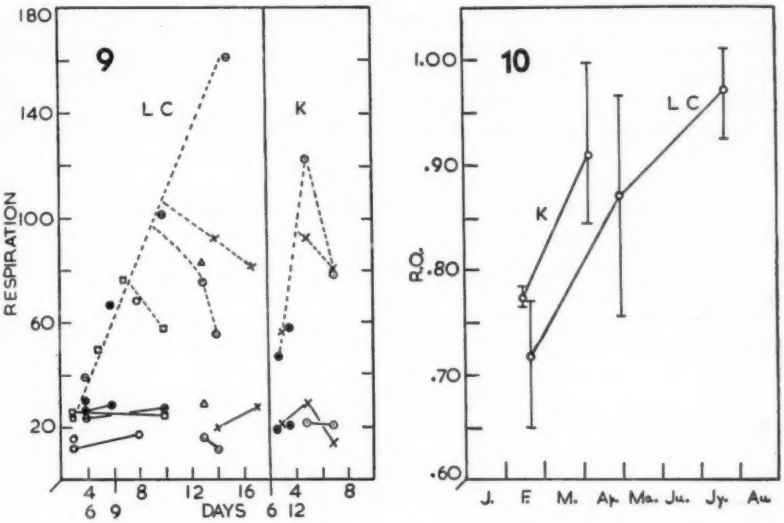


FIG. 9. The drifts in respiration at rust infections (Race 15B) on the first leaves of Little Club (LC) and Khapli (K). Broken lines, infected leaf-pieces. Solid lines, uninfected leaf-pieces. Ordinate: microliters of oxygen consumed per 1 mgm. of tissue nitrogen per hour. Abscissa: days after inoculation. Note that the time scales for Expts. I.L. and I.K. have been telescoped. See footnotes to Tables VIII and X. Symbols: as for Fig. 5. Primary data are given in Tables VIII and X.

FIG. 10. The seasonal drift in the respiratory quotient of disks cut from the first leaves of Little Club (LC) and Khapli (K). Ordinate: R.Q. Abscissa: months of the year. Primary data are given in Tables VIII and X.

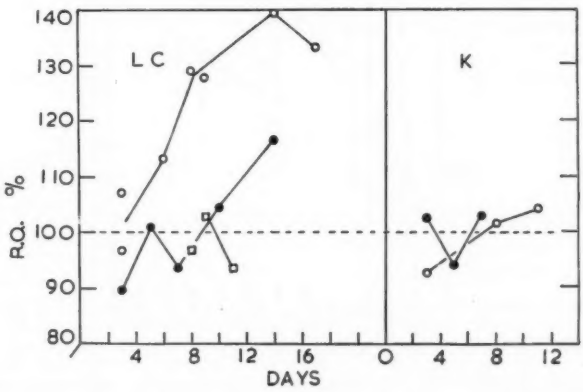


FIG. 11. The drift in the respiratory quotient at rust infections (Race 15B) on the first leaves of Little Club (LC) and Khapli (K). Ordinate: R.Q. as a percentage of that for uninfected leaves. Abscissa: days after inoculation. Symbols: ○ Expts. I.L., II.L., and III.L.; ● Expts. IV.L. and V.L.; □ Expt. VII.L.; ○ Expt. I.K.; ● Expt. IIa.K. Primary data are given in Tables VIII and X.

quotient and carbohydrate content (Fig. 12). It should be added that iodine tests revealed no trace of starch formation at the infections at any time during Experiment IV.L. with Little Club and that there was actually less total soluble sugar in the rusted than in the healthy leaves. As mentioned above, the respiratory rise in this experiment was among the lowest recorded in any

TABLE XI

CARBOHYDRATE ANALYSES FOR EXPERIMENTS IV.L. AND II*a*.K.

Expt.	Variety	Days after inoculation	Sucrose*		Hexose*		Total sugar*	
			H	R	H	R	H	R
IV.L.	Little Club	3	5.07	2.06	1.53	0.52	6.60	2.58
		5	1.54	2.17	0.70	1.04	2.24	3.21
		7	4.71	1.98	1.28	1.34	5.99	3.32
		10	0.47	0.62	0.54	0.64	1.01	1.26
II*a*.K.	Khapli	3	3.07	2.58	0.47	2.49	3.54	5.07
		5	—	5.19	—	1.62	—	6.81
		7	0.32	0.45	1.00	0.41	1.32	0.86

**Expressed as hexose % dry weight.*

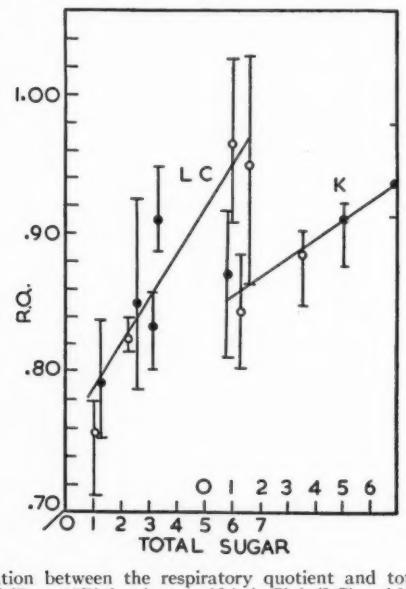


FIG. 12. Correlation between the respiratory quotient and total sugar content in healthy and infected (Race 15B) first leaves of Little Club (LC) and Khapli (K). Ordinate: R.Q. Abscissa: total sugar (as hexose) in per cent of total dry weight. Symbols: ● infected leaves; ○ uninfected leaves. The regression equations for the lines in the figure are: for Little Club, $y = 0.03178x + 0.7588$ ($r = +0.865$, $P<0.01$); for Khapli, $y = 0.01352x + 0.8414$ ($r = +0.945$, $P<0.02$). Primary data are given in Tables VIII, X, and XI.

of the experiments with Little Club, being approximately one-third that observed in Experiment VIII.L. Turning now to the relationship between oxygen consumption and carbohydrate content, it was found that the four points for healthy Little Club leaves lay along a rectangular hyperbola. (The correlation coefficient (r) between the reciprocals of the respiration rate and total carbohydrate content was calculated to be $+0.97$. $P<0.05$.) For the present the same relationship may be assumed for Khapli. Moreover, it is clear that this relationship is in the main determined by the concentration of sucrose rather than hexose (Fig. 13). Fig. 13 also shows that in infected Little Club leaves, the oxygen consumption was positively correlated with hexose content, the correlation being more exact during the respiratory rise than after the peak was reached. Allen's (1) data for mildewed wheat also showed these relationships (see Fig. 14), as he pointed out. However, in his experiment the hexose concentration rose with respiration to a concentration four times that found in healthy leaves, while in Experiment IV.L. the hexose concentration in the infected leaves did not exceed that found in healthy ones. In infected leaves of Khapli, oxygen consumption was negatively correlated

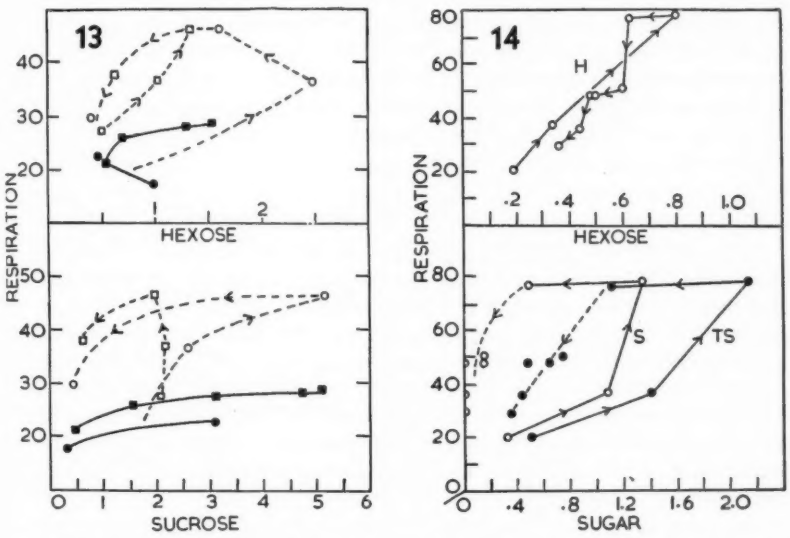


FIG. 13. The relationships between respiration and sugar content in healthy and infected (Race 15B) first leaves of Little Club and Khapli. Ordinate: microliters of oxygen consumed per 10 mgm. dry weight per hour. Abscissa: hexose (top) and sucrose (bottom) as hexose in per cent of dry weight. Symbols: ■ uninfected Little Club; □ infected Little Club; ● uninfected Khapli; ○ infected Khapli. Arrows indicate the direction of drifts in infected leaves. Primary data are given in Tables VIII, X, and XI.

FIG. 14. The relationships between respiration and hexose (H), sucrose (S), and total sugar (T.S.) content in mildewed wheat leaves. Replotted from the data of Allen (1). Ordinate: microliters of oxygen consumed per 100 mgm. fresh weight per hour. Abscissa: hexose (top) and sucrose and total sugar (bottom) all as hexose in per cent of fresh weight. Arrows indicate the direction of drifts.

with hexose content during the respiratory rise (Fig. 13). In both infected Little Club and Khapli the fall in respiration from the peak was correlated with a fall in sucrose content (Fig. 13). Although Allen stated that the respiration of mildewed wheat leaves showed no relation to sucrose content or to total carbohydrate the data given in his Figs. 17 and 18 and replotted here as Fig. 14 show that the decline from the respiratory peak was accompanied by a marked drop in sucrose and that the hexose content only fell later. Moreover, during the decline, respiration was well correlated with a decrease in total carbohydrate (Fig. 14) as reported by Krotkov (10) for starving wheat leaves.

Thus consideration of Figs. 13 and 14 leads to the conclusion that the increase in respiration in tissue under attack by *Erysiphe* or *Puccinia* is not initially dependent on a rise in sucrose or hexose content above the amounts present in healthy tissue. Although such a rise does occur under suitable conditions and is essential for optimal development of the parasites, it seems that infection also involves a transition to a new relationship, in which a given level of carbohydrate corresponds to a greater rate of respiratory oxidation than in comparable healthy tissue. The duration and extent of the respiratory rise are, however, determined by availability of carbohydrate (see also Fig. 9). These data, both Allen's and that presented here for rust, suggest that more extensive work may show that the rates of respiration in healthy wheat leaves and during the decline from the respiratory peak in infected leaves are related to sucrose concentration by a curve which approaches a rectangular hyperbola as reported for the potato tuber (4).

Discussion

The bulk of the respiratory increase caused by *Erysiphe* is due to a stimulation of the respiration of the tissue under attack (2). It is not practicable to separate the mycelium of the rust fungus from the tissues of its hosts and the measurements reported in this paper were made using small disks or pieces of infected leaves. It is, however, considered that the mycelium of the rust fungus is not directly responsible for all the observed alterations in respiration, dry weight, and nitrogen content, but rather that major changes occur in the host tissue at and in the environs of infections. This view is consistent with the occurrence of hypertrophy at rust infections on susceptible hosts (20).

The increase in respiration, like the accumulation of radioactive sugars (Figs. 3 and 4) and other substances (15), was localized at the sites of infections. The values of the respiratory quotient and the rise in the respiratory quotient which occurs at type 4 infections under conditions which have been described (p. 612) suggest that carbohydrate is the main respiratory substrate at rust infections, at least during the respiratory rise. These conclusions are in general agreement with previous findings for mildew on wheat (1, 2).

For each of the two species Khapli and Little Club the duration of the respiratory rise at infections of stem rust appeared to be dependent on the

environmental conditions, particularly illumination, during the development of the disease (Fig. 9). Heaviness of inoculation is probably a factor here also, since evidence of competition between infections was obtained (Table III). The rate of rise of the respiration, expressed per unit of tissue nitrogen, was however remarkably constant for comparable infections on Little Club (Fig. 9). Heavily-infected leaves of Khapli quickly turned yellow and underwent rapid and marked losses in dry weight and total nitrogen (Figs. 5 and 6). These phenomena suggest a rapid proteolysis followed by transport of the soluble nitrogen to competing sinks in other parts of the growing plants. By contrast, there were gains in dry weight and in total nitogen per unit area at infections on Little Club (Figs. 5 and 6). Total nitrogen per unit dry weight remained almost constant for 10 or more days. Thus when the respiration rates on a dry weight or nitrogen basis are compared for the two species it is seen that the rate of the respiratory increase in Khapli was greater than that in Little Club; moreover, the respiratory peak and the secondary decline in respiration occurred sooner in Khapli than in Little Club (Figs. 8 and 9).

When changes in dry weight and nitrogen are neglected and the comparisons made on the basis of unit area, it is seen that, under comparable conditions, the respiration rises at approximately the same rate on each of the three 'varieties', Khapli, Khapli I, and Little Club, which are respectively resistant, moderately susceptible, and susceptible (Fig. 1). (In passing it may be noted that the data for experiment II*a*.K. suggest that even on an area basis the respiration rate may rise more quickly in infected Khapli leaves.) The duration and therefore also the amount of the respiratory rise were, however, greater the more susceptible the host and the fact that the results for the off-type Khapli, here designated Khapli I, were intermediate between those for the two dissimilar species, Khapli and Little Club, increases the significance of comparative studies on the latter. The results obtained with mildew on two closely related resistant and susceptible varieties of barley were quite different from those with rust in that the rate of increase of respiration was much less on the resistant variety (Fig. 2). Clearly much more extensive data will be required before any general pattern of the relationship between the rate of the respiratory rise of infected tissue and the resistance or susceptibility of the tissue can safely be assumed for either of the two parasites *Puccinia* and *Erysiphe*. Clearly also, changes in dry weight and nitrogen content should be taken into account. Protein nitrogen should also be determined.

On graphical analysis (Figs. 13 and 14), the data relating carbohydrate content and the respiration rate of rusted wheat leaves, as well as the similar data of Allen (1) for mildewed wheat leaves, suggest that the establishment of infections involves a transition to a new relationship in which a given level of carbohydrate corresponds to a greater level of oxygen consumption than in comparable healthy tissue. It has already been pointed out that, per unit of oxygen consumed, the No. 1 carbon atom of glucose, supplied exogenously, is oxidized to carbon dioxide faster by rusted than by healthy tissue (15). Experiments in which glucose-1-C^{14} and uniformly labelled glucose were fed

separately to samples of both healthy and rusted or mildewed leaf tissue have now shown that the relative contribution of the No. 1 carbon atom of glucose to the carbon dioxide evolved rises with the progress of development of both mildew and rust. One explanation of these observations is that, in the diseased tissue, there is an increase in the proportion of the respired carbon which passes through an 'alternative' respiratory pathway, possibly the 'hexose monophosphate shunt'. Experimental data bearing on this point and a discussion of mechanisms possibly responsible for the respiratory increase will be presented in Part III (16).

In the light of these findings, and of the differences in the rate of rise of respiration at infections on Khapli and Little Club, it should be borne in mind that the rate of increase in respiration and the balance or relative rates of alternative pathways in the host tissue at infections may be of the utmost importance in determining the success or failure of the parasite, particularly if such rates affect the efficiency and rates of protein synthesis and breakdown. These ideas, though speculative, appear to open a fruitful field for further work, even if the adjustment of metabolic systems involved in successful obligate parasitism should ultimately prove to be on a far more subtle level.

Acknowledgments

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References

1. ALLEN, P. J. Changes in the metabolism of wheat leaves induced by infection with powdery mildew. Am. J. Botany, 29 : 425-435. 1942.
2. ALLEN, P. J. and GODDARD, D. R. A respiratory study of powdery mildew of wheat. Am. J. Botany, 25 : 613-621. 1938.
3. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis. 6th ed. A.O.A.C. Washington. 1945.
4. BARKER, J. Analytic studies in plant respiration. VI. The relation of the respiration of potatoes to the concentration of sugars and to the accumulation of a depressant at low temperatures. Part 3. The relation of the respiration to the concentration of sucrose. Proc. Roy. Soc. B, 119 : 453-473. 1936.
5. FLOR, H. H. Inheritance of rust reaction in a cross between the flax varieties Buda and J.W.S. J. Agr. Research, 63 : 369-388. 1941.
6. FLOR, H. H. Inheritance of pathogenicity in *Melampsora lini*. Phytopathology, 32 : 653-669. 1942.
7. FLOR, H. H. Genetics of pathogenicity in *Melampsora lini*. J. Agr. Research, 73 : 335-357. 1946.
8. FLOR, H. H. Inheritance of reaction to rust in flax. J. Agr. Research, 74 : 241-262. 1947.
9. GASSNER, G. and FRANKE, W. Untersuchungen über den stickstoffhaushalt. rostinfizierter getreideblatter. Phytopathol. Z. 5 : 517-570. 1938.
10. KROTKOV, G. Carbohydrate and respiratory metabolism in the isolated starving leaf of wheat. Plant Physiol. 14 : 203-226. 1939.
11. NEISH, A. C. Analytical methods of bacterial fermentations. Report No. 46-8-3 (2nd revision), National Research Council, Ottawa, Ontario. 1952.

12. SCHALLER, C. W. The effect of mildew and scald infection on yield and quality of barley. Agron. J. 43 : 183-188. 1951.
13. SCOTT, T. A. and MELVIN, E. H. Determination of dextr n with anthrone. Anal. Chem. 25 : 1656-1661. 1953.
14. SEMPIO, C. Metabolic resistance to plant diseases. Phytopathology, 40 : 1-23. 1950.
15. SHAW, M. and SAMBORSKI, D. J. The physiology of host-parasite relations. I. The accumulation of radioactive substances at infections of facultative and obligate parasites including tobacco mosaic virus. Can. J. Botany, 34 : 389-405. 1956.
16. SHAW, M. and SAMBORSKI, D. J. The physiology of host-parasite relations. III. To be published.
17. WYKES, G. R. An investigation of the sugars present in the nectar of flowers of various species. New Phytologist, 51 : 210. 1952.
18. WYLAM, C. B. Analytical studies on the carbohydrates of grasses and clovers. IV. Further developments in the methods of estimation of mono, di and oligo-saccharides and fructosan. J. Sci. Food Agr. 5 : 167-172. 1954.
19. YARWOOD, C. E. and CHILDS, J. F. L. Some effects of rust infection on the dry weight of host tissues. Phytopathology, 28 : 723-733. 1938.
20. YARWOOD, C. E. and COHEN, M. Hypertrophy from the uredial stage of bean rust. Botan. Gaz. 112 : 294-300. 1951.

MORPHOLOGICAL AND HISTOLOGICAL MODIFICATIONS OF PINE SEEDLINGS INDUCED BY PETROLEUM NAPHTHA[1]

By J. D. LaCroix[2] and A. T. Guard[3]

Abstract

Pinus caribaea, *P. sylvestris*, and *P. resinosa* seedlings at various stages of growth were subjected to pre-emergence and postemergence treatments with Standard Weed Killer, a Stoddard solvent naphtha, at the rate of 50 to 1000 gal. per ac. Structural responses were a darkening and wilting of the cotyledons and hypocotyl in young seedlings and a yellowing and burning of the older foliage. Outstanding histological modifications were plasmolysis and cytolysis of the cortex, pith, and mesophyll and stomatal closure. Anatomical variation in pine seedlings is suggested as an explanation for species and organ differences in response to the petroleum spray. Less mature seedlings were more susceptible to the Stoddard solvent than older seedlings. Penetration, once the initial stages had been passed, was rapid, though limited in mature tissues by the thick-walled endodermis. The oil passed through the stomata and moved into the intercellular spaces. Once in the cell, the oil combined with the cell contents causing plasmolysis followed by cytolysis.

Since conifer seedlings grow very slowly during their first two years, they are constantly subjected to injury from mechanical cultivation equipment and hand weeding. Until recently, hand weeding was a common practice, but present day rises in labor costs have made this an expensive operation. Eliason (8) and Stoeckeler (19) reported exceptional savings in weeding costs with the use of oil sprays such as Sovasol, Varsol, Stanisol, Stoddard solvent, stove oil, and mineral spirits. On occasion, however, they injure the seedlings, especially if the rate of application is high enough. Since these effects are variable it was thought that a study of the nature of the detrimental effect on pine seedlings would offer helpful information in correcting the problems associated with the use of petroleum oils.

Thus far most of the work with petroleum sprays in conifer nurseries has not been of a fundamental nature. It appears that field tests solely have been emphasized even though there is a definite need for basic research. Up until this time, the killing action of oils has been given prime emphasis and the reasons for this action have been neglected. Morphological and anatomical studies involving penetration, extent and nature of translocation, and the histological effect of oils should bring the field testing and basic research into better balance as would a study of the growth stages and growth conditions before and after treatment.

Even though numerous papers have been written concerning the utilization of oils on angiosperms, those involving conifers have dealt with the problem mainly in a practical and morphological sense, notably those by Clements and

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Eliason (4), Robbins *et al.* (16), Vidme (21), Stoeckeler (18, 19, 20), Day (7), Eliason (8), Grigsby *et al.* (11), Bouvarel (2), Brooks (3), Cossitt (5), Farrer (9), Havis (13), Srivastava (17), and Faulkner (10). Woodford and Ivens (22) compiled an extensive review on the chemical methods for control of weeds in forest nurseries and Norman *et al.* (15) thoroughly reviewed the use of oil sprays as herbicides.

In the present investigation the difference in response of three species of *Pinus* to petroleum naphtha was studied. The growing point, cotyledons, hypocotyl, and leaves were examined morphologically and histologically and correlations between stages or conditions of growth and the susceptibility of the seedlings to injury by petroleum naphtha were determined.

Materials and Methods

Three species of pine, *Pinus caribaea* More. (slash pine), *P. sylvestris* L. (Scotch pine), and *P. resinosa* Ait. (red pine) were grown in the greenhouse in 6-in. clay pots. Periodic morphological observations were made of the sprayed plants. Material from the treated plants and from control plants was collected periodically for histological study. Material was killed and fixed in formalin – alcohol – acetic acid, dehydrated, embedded by the butyl alcohol – paraffin method, and sectioned at 15 μ. Safranin and fast green were the stains used.

Either the plants or soil were sprayed with undiluted Standard Weed Killer, supplied by the Standard Oil Company. It is a Stoddard solvent naphtha—a petroleum fraction consisting of a mixture of hydrocarbons. The oil, with a boiling range of 300°–400° F., is primarily paraffinic in nature, but does contain about 10% aromatics. The spray was applied with a Hudson hand sprayer with an adjustable nozzle. A computed amount of spray was directed on the soil in each pot taking into consideration its soil area.

Both pre-emergence and postemergence treatments were made. In the pre-emergence treatments and in those treatments involving seedlings possessing seed coats, 100 seeds per pot were sown, of which approximately 45% were germinable. The rates of application of Standard Weed Killer material used ranged from 50 to 1000 gal. per ac. The seedlings to be subjected to pre-emergence treatment were divided into two groups, those sprayed one day after they were sown and those treated approximately 10 days after they were sown. The growing point, cotyledons, and hypocotyl were collected 2 and 30 days after treatment.

In the postemergence studies, the plants were sprayed in the following three stages of growth: (*a*) fully emerged seedlings which still retained their seed coats; (*b*) seedlings five to seven months old; (*c*) one-year-old seedlings. The growing point, cotyledons, hypocotyl, and leaves were collected 1, 7, and 28 days after the spray application.

Following a method employed by Knight *et al.* (14) the penetration of Standard Weed Killer into pine leaves was investigated. This involved the use of water soluble pyridine in aqueous solution as a carrier for a fat stain

such as Sudan IV which is soluble in pyridine. The young leaves on one-year-old Scotch pine were selected to study the penetration, the oil being applied to the entire leaf by means of a small brush. Material was collected at intervals of 1, 24, 48, and 72 hr. after treatment. Leaves treated with pyridine were sectioned at 50 μ on the freezing microtome.

Observations

The young seedlings that had been sprayed one day after they were sown emerged very slowly and with difficulty even at the lower rates of application. There was no evidence of bending or twisting in the cotyledons or hypocotyl. The treated seedlings, rather than emerging with seed coats, often appeared with seed coats lacking. On occasion, the seed coats were retained for considerable lengths of time and were still quite evident six months after treatment. The hypocotyl, and in some cases the primordial leaves, remained short giving the plants a squatty appearance; even the cotyledons were not as expanded as in control plants. Very few secondary needles were evident even after six months. Those seeds that had received an application of 200 gal. per ac. just prior to emergence of the seedlings continued to germinate but germination was 20% less than the control planting (Fig. 1). However, this percentage of germination was higher than that obtained when seeds in pots received an equal amount of spray one day after they were planted.

The response of slash pine seedlings possessing seed coats was more rapid and severe than of similar seedlings of Scotch pine and red pine (Table I). Within one hour plants darkened to such an extent that the cotyledons and hypocotyl appeared saturated with oil. This was almost simultaneously followed by a bending of the hypocotyl at, or below, the growing tip. The cotyledons and the hypocotyl of all damaged plants appeared flaccid and shrivelled. Although growth was slightly retarded in the plants that had escaped serious injury, these plants did possess secondary, or adult fascicled, needles and would probably continue to grow.

In seedlings five to seven months old, injury was most severe in slash pine. Although the responses in the older plants were slower, the needles eventually

TABLE I

EFFECT OF POSTEMERGENCE TREATMENTS OF STANDARD WEED KILLER ON *P. caribaea*, *P. sylvestris*, AND *P. resinosa* SEEDLINGS POSSESSING SEED COATS

Treatment, gal. per ac.	Mortality (%) in seedlings 13 days after spray		
	P. caribaea	*P. sylvestris*	*P. resinosa*
Control	0	0	0
50	45.5	0	0
100	46.7	0	0
200	61.5	10.3	0
600	91.7	17.6	4.2

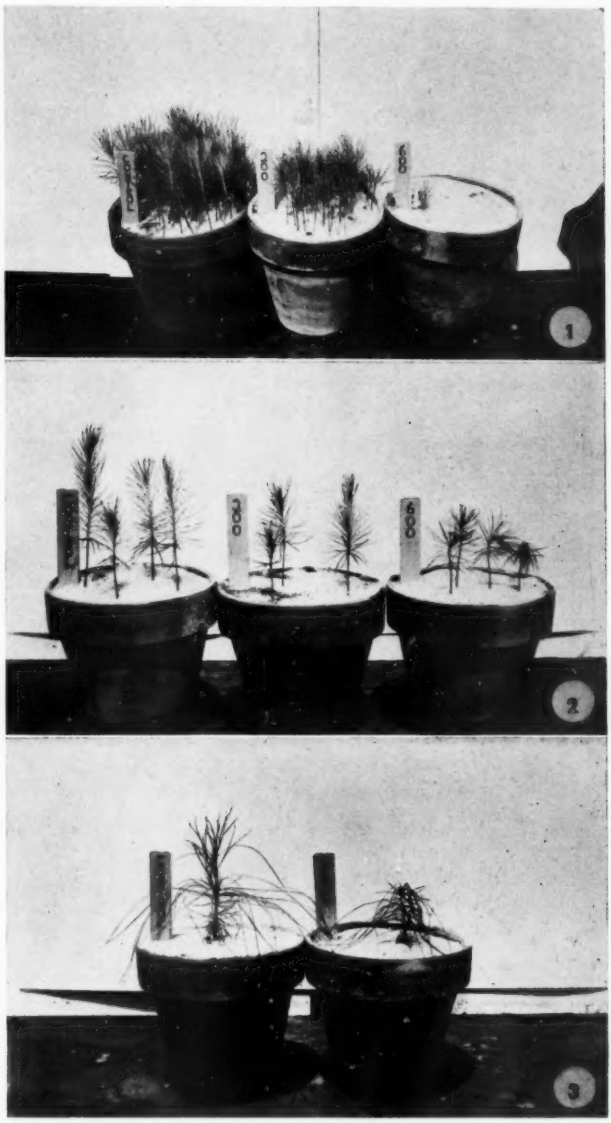
became chlorotic; they became limp and those at the growing tip drooped. The latter became more flexible and in extreme cases there was bending in this region (Fig. 2). The young leaves which were severely damaged ultimately turned brown and died as did the older lower leaves which exhibited oil burning.

One-year-old pine seedlings were slower in response and more tolerant of Standard Weed Killer than younger seedlings. Secondary needles when young were much more susceptible to damage than older needles. Where needles were present in fascicles, marked damage and burning occurred to the leaves at the sheath, presumably owing to the collection of oil droplets around the sheath. Hence, the needles drooped from the point of injury (Fig. 3). The initial injury was followed by the more mature foliage becoming chlorotic and ultimately burning at the tips of the needles. The very young shoots and terminal buds were particularly sensitive to the oil and growth was arrested.

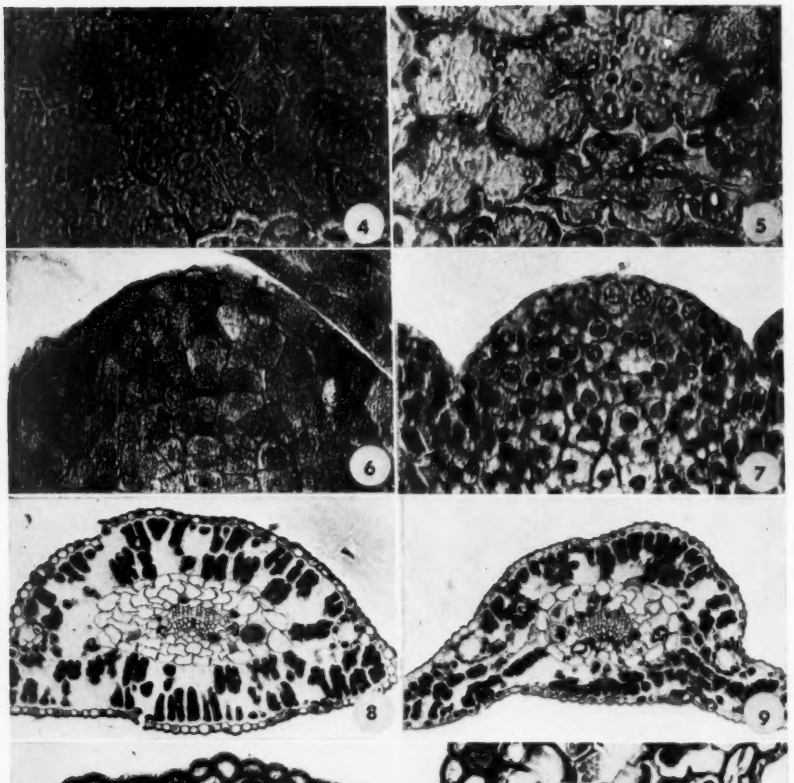
At the expiration of one hour, the petroleum naphtha covered the cutinized epidermal cells, gathered in any depressions between the cells, and collected around the subsidiary and guard cells of the stomata. After passing through the latter the oil travelled into the intercellular spaces between the chlorenchyma cells. Within 48 hr. the naphtha spray had progressed to the thick-walled endodermal cells. Soon thereafter, the oil entered the chlorenchyma cells and existed in the form of minute droplets (Figs. 4, 5); these varied in number from 1 to 10 per cell. The damage was further characterized by a contraction or shrinkage of the entire cell including the cell wall. This ultimately led to the enlargement of the intercellular spaces. Cellular injury most generally took the form of cytoplasmic and nuclear disorganization which eventually resulted in complete cytolysis.

The histological response of young tissue to pre-emergence treatment was rapid and the injury was severe. The most responsive tissues were the epidermis, cortex, and pith. The cytoplasm of the cells of the various zones of the apical meristem and of the leaf primordia became less dense or less granular giving the cells a vacuolated appearance. The nucleus contracted and was prominent as compared to that in the control (Figs. 6, 7). In the hypocotyl the cells of the cortex and pith became vacuolated thus giving the section a translucent appearance.

The most outstanding response to the postemergence application was in the form of a severe cytolysis and breaking down of cells of the cortex, pith, and mesophyll. In organs possessing an endodermis, a very noticeable lack of injury was evident beyond this thick-walled layer of cells. The epidermal cells of the hypocotyl were misshapen and the cell contents plasmolyzed. In addition to the deformed epidermis, there was a drastic plasmolysis of the cortical parenchyma cells. The thin-walled secretory epithelial cells and the thickened fibers lining the resin ducts were severely misshapen. Still further injury was evidenced by the crushed appearance of the phloem and cytolysis of the pith.



FIGS. 1–3. Morphological structure of seedlings of untreated plants, and of plants treated prior to emergence, five months and one year old, with Standard Weed Killer. FIG. 1. *P. caribaea* treated immediately prior to emergence with 200 and 600 gal. per ac. FIG. 2. Five-month-old *P. caribaea* treated with 200 and 600 gal. per ac. FIG. 3. One-year-old *P. caribaea* after treatment with 1000 gal. per ac.



FIGS. 4–11. Anatomical structure of leaves and stem tips of untreated and treated plants. FIG. 4. Cross-section of untreated leaf of *P. sylvestris* with normal mesophyll tissue. FIG. 5. Cross-section of leaf 48 hr. after treatment showing oil droplets within contracted chlorenchyma cells, large intercellular spaces, cytoplasmic and nuclear disorganization, and less evident nuclei. FIG. 6. Longitudinal section of normal stem tip of *P. caribaea*. FIG. 7. Longitudinal section of stem tip subjected to pre-emergence sprays showing vacuolation of cells of apical meristem and contracted nuclei. FIG. 8. Cross-section of seven-month-old untreated primordial leaf of *P. caribaea*. FIG. 9. Cross-section of treated seven-month-old primordial leaf showing partial collapse and distortion of leaf shape, distortion of mesophyll tissue, including resin ducts and closed stomata. FIG. 10. Cross-section of one-year-old normal leaf of *P. sylvestris*. FIG. 11. Cross-section of one-year-old treated leaf exhibiting stomatal closure, damaged mesophyll tissue, and resin ducts. Note translucent epidermal and hypodermal layers.

The most apparent effect of the petroleum naphtha on seven-month-old primordial leaves and one-year-old needles was a partial collapse and distortion of leaf shape. This distortion appeared to be caused by the shrinkage of the mesophyll tissue; pronounced protoplasmic changes were noted in the chlorenchyma cells, the secretory epithelial cells, and fibers. Partial or complete stomatal closure was evident (Figs. 8, 9, 10, 11).

Discussion

Although such rates of application of Standard Weed Killer as 200, 600, and 1000 gal. per ac. are well above the amount applied in nursery practice, they did induce sufficient morphological and histological modifications for a study of their nature. The severe injury resulting from such heavy applications indicates the damage that can result from the careless use of such sprays. Eliason (8) and Stoeckeler (19) recommended dosages of 20 to 150 gal. per ac. depending on the age of the seedlings and the type of treatment. The principal method of application of petroleum oils in coniferous nurseries has been as a postemergence spray. Faulkner (10) reported successful pre-emergence treatment and our findings indicate that although germination may be decreased by rates of 200 gal. per ac., the control of weeds would be sufficient to compensate for planting a higher number of seeds.

The authors established that less mature tissues are more susceptible, but in pre-emergence studies the younger tissues responded less rapidly possibly because Standard Weed Killer is a contact herbicide. The retention of seed coats following pre-emergence treatments suggested an inhibited elongation of the cotyledons.

The morphological responses were generally similar to those reported by Stoeckeler (18, 19, 20), Eliason (8), and Cossitt (5), all of whom noted chlorosis and burning of the foliage, particularly near the base of the needles. The histological effects observed in the needles, a breakdown of the physical organization of the protoplasm followed by a collapse and shrinkage of the cell, eventually resembled a portion of the leaf structure described in guayule seedlings by Addicott (1) who reported cytolysis of the epidermis and palisade tissues. Contrary to the observations of Eliason (8), data from this investigation indicated that cotyledons and primary needles were no more resistant than young secondary needles. Actively growing regions and immature tissues such as young secondary leaves and needles at the sheath were subject to spray injury. The primary effects were most marked in the cotyledons, hypocotyl, and young primordial leaves.

Numerous workers have described generic differences in response to petroleum oils and this present investigation has indicated that slash pine is more susceptible to the spray than Scotch or red pine. Such species differences between slash, Scotch, and red pine may be ascribed to the more rapid growth rate of slash pine or the presence of more active tissues.

Differences in leaf anatomy and in degree of development of particular tissues may be responsible for species and organ differences in response to the

spray. It is significant that of the three species of pine studied, slash pine possesses the least number of resin ducts and exceeds Scotch and red pine in the number of stomata. Furthermore, the hypocotyl and cotyledonary tissues are relatively immature so that greater penetration may be possible, while the leaves examined had mature tissue such as the thick-walled endodermis which might make it more difficult for the oil to penetrate these structures. A thinner cuticle in less mature tissues would also account for greater penetration of Standard Weed Killer. In the studies involving the penetration of oil it appeared that the endodermis limited the further penetration of the petroleum naphtha. The lesser degree of development of this layer of cells may account for the rapid morphological and anatomical responses in cotyledons and hypocotyl, thus allowing the herbicide access to the xylem and phloem, which if injured would result in the death of the plant. Judging from this study it is not presumptuous to state that because of the well-developed, thick-walled endodermis the stelar tissues were not invaded by the oil.

The exact mechanism of the toxic action of petroleum oils on plants is still a matter of speculation. The authors believe that the oil entered the cell and in passing through the cell membrane altered its permeability or destroyed it. The oil combined with the cell contents causing a plasmolysis followed by a cytolysis of the cell constituents. This reaction permitted water to escape from the cells and this ultimately led to desiccation of the plant tissue. Havis (12) felt that the resistance to injury to petroleum hydrocarbons may be associated with the presence of essential oils or oil ducts. It is interesting to note that of the three species investigated in this study, slash pine possesses the least number of resin ducts. Crafts *et al.* (6) stated that the possession of oil ducts will not serve to explain the resistance of plants to oil sprays.

References

1. ADDICOTT, F. T. Anatomical effects of oil spray injury in guayule seedlings. Phytopath. 34 : 697-699. 1944.
2. BOUVAREL, P. Le désherbage chimique des pépinières. Rev. forestière franç. 525-527. 1951. (From Forestry Abstr. 13. No. 1146. 1951.)
3. BROOKS, R. O. Oil spray weeding as applied to forest nurseries in Pennsylvania. Tree Planters' Notes, 4 : 6-7. 1951.
4. CLEMENTS, C. M. and ELIASON, E. J. Notes on weed control in coniferous seedbeds. Mimeo. by N.Y. State Conservation Dept. 1947.
5. COSSITT, F. M. Use of petroleum products as selective herbicides in Southern pine seedbeds. J. Forestry, 49 : 773-775. 1951.
6. CRAFTS, A. S., CURRIER, H. B., and LEONARD, O. A. Toxicity of herbicidal oils. Proc. 3rd World Petroleum Congress. 198-203. 1951.
7. DAY, M. W. Use of mineral spirits for control of weeds in evergreen nurseries. Research Rept. 6th Ann. N. Cent. Weed Control Conf. 220. 1949.
8. ELIASON, E. J. The use of oil sprays for the control of weeds in coniferous nurseries—1948 supplement. Mimeo. by N.Y. State Conservation Dept. 1949.
9. FARRER, R. P. The use of selective herbicides in forestry in general, and in nursery work in particular. Empire Forestry Rev. 30 (1) : 66-71. 1951.
10. FAULKNER, R. Notes on nursery irrigation and on chemical weed control practices in the U.S.A. and Canada. Forestry, 25 (2) : 126-134. 1952.
11. GRIGSBY, B. H., CHURCHILL, B. R., HAMNER, C. L., and CARLSON, R. F. Chemical weed control. Mich. Agr. Expt. Sta. Circ. Bull. 214. 1949.

12. HAVIS, J. R. The herbicidal properties of petroleum hydrocarbons. Cornell Agr. Expt. Sta. Memoir, 298 : 1-20. 1950.
13. HAVIS, J. R. Chemical weed control in nurseries. Am. Nurseryman, 93 (5) : 13. 1951.
14. KNIGHT, H., CHAMBERLIN, J. C., and SAMUELS, C. D. On some limiting factors in the use of saturated petroleum oils as insecticides. Plant Physiol. 4 : 299-321. 1929.
15. NORMAN, A. G., MINARIK, C. E., and WEINTRAUB, R. L. Herbicides. Ann. Rev. Plant Physiol. 1 : 141-168. 1950.
16. ROBBINS, P. W., GRIGSBY, B. H., and CHURCHILL, B. R. Report on chemical weed control for conifer seedlings and transplants. Mich. Agr. Expt. Sta. Quart. Bull. 30 : 237-240. 1947.
17. SRIVASTAVA, T. N. The application of selective herbicides to forestry practice. Indian Forester, 77 (3) : 176-191. 1951. (From Forestry Abstr. 13. No. 1145. 1951.)
18. STOECKELER, J. H. Killing nursery weeds with oil sprays. Lake States Forest Expt. Sta. Tech. Notes, 290. 1948.
19. STOECKELER, J. H. Control of weeds in conifer nurseries by mineral spirits. Lake States Forest Expt. Sta. Paper No. 17. 1949.
20. STOECKELER, J. H. Hydrocarbon content and storage period affect toxicity of mineral spirits used as selective herbicides for red pine nursery beds. J. Forestry, 49 : 647-649. 1951.
21. VIDME, T. Kjemisk ugrastyning. Tidsskr. Skogbruk. 56 : 287-296. 1948. (From Forestry Abstr. 11. No. 3263. 1950.)
22. WOODFORD, E. K. and IVENS, G. W. Chemical methods for the control of weeds in forest nurseries. Forestry Abstr. 12 : 139-143. 1950.

CHROMOSOME SUBSTITUTION IN HEXAPLOID WHEAT[1]

By John Unrau,[2] Clayton Person,[3] and John Kuspira[4]

Abstract

The procedures involved in the various phases of chromosome substitution in common wheat are briefly outlined and explained. Complications encountered with reciprocal translocations are clarified. The following subjects are discussed: development of chromosome-deficient series in other varieties, transfer of single chromosomes from donor varieties to chromosome-deficient lines to develop substitution lines, alien substitutions, and combination of two chromosome substitutions into a single line. There is a brief discussion of the value of the chromosome substitution method especially in the study of gene dosage and interaction as affecting certain characters.

Introduction

Chromosome substitution in hexaploid wheat, i.e. the transfer of single chromosome pairs from one variety to the next, is now possible because there are available all 21 nullisomic or monosomic aneuploid lines in the variety Chinese Spring (8, 9, 10). The usefulness of nullisomic analyses in wheat for associating genes with specific chromosomes and for studying the complex genetic control of certain characters and the unusual action of certain types of genes has been clearly shown by a number of workers (Sears (8, 9), Larson (4), and Unrau (12)). Recently Sears (10) has briefly described certain aspects of the procedures involved in making chromosome substitutions and in the present report certain phases of the procedures described by him are elaborated and others hitherto not reported are briefly described.

Since the method described is based on relatively new, exacting, and in some instances, complicated procedures, it is hoped that clarity and usefulness has not been sacrificed for brevity. The report will be useful to those who wish to evaluate the method as well as to those who contemplate using it. The reader should realize that some phases of the procedures will be modified as more investigators work with aneuploids. Indeed, departures from the procedures described here may be advantageous in certain instances.

Methods

1. The Development of Chromosome-deficient Series in Other Varieties

For certain genetic analyses, and certainly for improvement programs utilizing chromosome substitutions, the chromosome-deficient series of the variety Chinese Spring cannot be used directly. It is necessary, then, to develop the chromosome-deficient series in those varieties that are to form the

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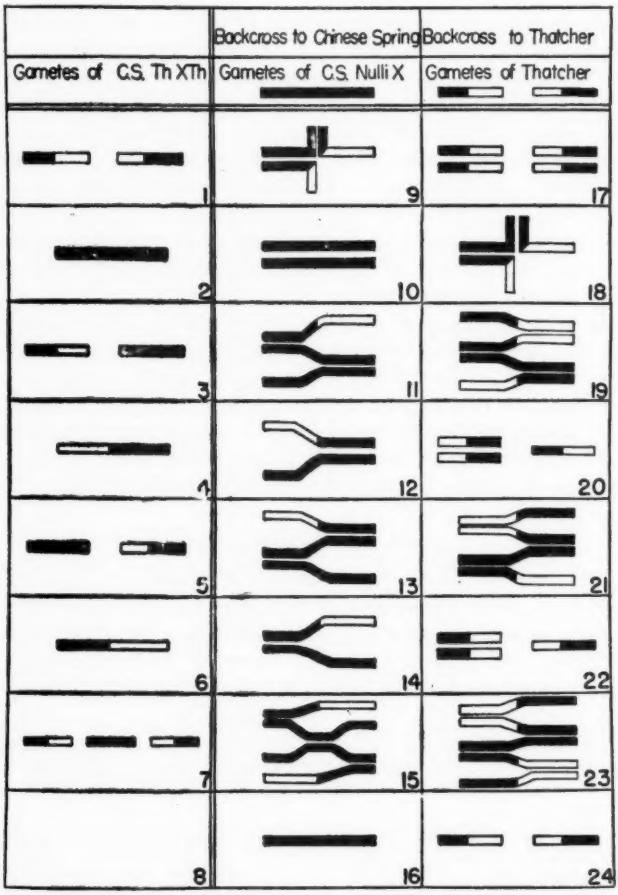
basis of future studies and improvement. At the University of Alberta the aneuploid series have been or are being developed in the hard-red-spring variety Thatcher and in the soft-white-spring variety Lemhi. The procedure of developing such deficient lines is described below using the chromosome-I line as an example. It is assumed that this chromosome of Thatcher is not involved in a translocation with respect to chromosome I of Chinese Spring.

Step 1. Chinese Spring nulli- or mono-I ♀ is crossed with Thatcher ♂.

Step 2. The monosomic ♀ plants of the F_1 are crossed with Thatcher ♂.

Step 3. Monosomic progeny in each backcross generation are backcrossed to Thatcher until the genotype of Thatcher has been sufficiently reconstituted. After six backcrosses, the chromosome-I-deficient line will have, theoretically, over 98% of the genotype of Thatcher.



The presence of translocations between the varieties concerned introduces some complications. Sears (10) has shown that Thatcher and Chinese Spring differ by a reciprocal translocation involving chromosomes IV and X. Taking Chinese Spring as the standard, the chromosomes of Thatcher involved in the translocation can be designated X–IV and IV–X. If Chinese nulli-X is used as the female parent in the transfer of this deficiency to Thatcher, the transmission of the deficiency through the monosomic F_1 to the backcross generations is as described in Fig. 1.

The behavior of the three chromosomes at meiosis in the monosomic F_1 and backcross plants is of prime importance in determining the chromosomal constitution of the progeny derived from each successive backcross. If pairing and crossing over occur regularly in the translocated segments, a chain of three will likely persist until the first metaphase. In this situation, and assuming consistently alternate disjunction, the progeny of the successive backcrosses would have the constitution as illustrated at 9 in Fig. 1. The final derived monosomic would be monosomic for chromosome X as this chromosome exists in the standard, Chinese Spring.

There are, however, several alternatives that must be kept in mind. The three chromosomes may be present with varying frequencies as a chain of three, as a bivalent and a univalent, or as three univalents. Under these conditions six additional types of gametes may be produced and progenies of types shown at 11 and 16 (Fig. 1) can be expected when the plant is crossed with Thatcher. In this situation, it is important to observe that two additional types of monosomics, those illustrated at 12 and 14 of Fig. 1, are

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FIG. 1. The gametes that may be produced by a monosomic with a chain of three with chromosomes IV and X involved in a reciprocal translocation, and the offspring possible when these gametes combine with those of the backcross parents.

(Note: Solid rods refer to chromosome IV; open rods to chromosome X. *4a*, *4b* and *10a*, *10b* represent arms of chromosomes IV and X respectively. The illustrations show maximum possible associations which in some cases may not often be realized, and a small population of cells may need to be examined to distinguish one from another.)

1, 2. Produced with frequency comparable to frequency of chain formation.
1. 19^{I} + *4a 10b* + *10b 4a*.
2. 19^{I} + *4a 4b*.

3–6. Gametes produced when chain formation fails.
3. 19^{I} + *4a 10b* + *4a 4b*.
4. 19^{I} + *10a 4b*.
5. 19^{I} + *4a 4b* + *10a 4b*.
6. 19^{I} + *4a 10b*.

7, 8. Gametes produced when chain formation fails (probably with lowest frequency).
7. 19^{I} + *4a 10b* + *4a 4b* + *10a 4b*.
8. 19^{I}.

9–16. Progeny possible in backcross of monosomic F_1 ♂ to Chinese Spring nulli-X ♀ to develop a substitution line.
9. 19^{II} + ch. 3 as in F_1.
10. 19^{II} + 2 *4a 4b;* nullisomic X.
11.* 19^{II} + ch. 3; def. 10*a*, dup. 4*a*.
12. 20^{II}; def. 4*a* and dup. 10*a*.
13. 19^{II} + ch. 3; def. 10*b*, dup. 4*b*.
14. 20^{II}; def. 4*b*, dup. 10*b*.
15. 19^{II} + ch. 4; def. *10a* + *10b;* dup. *4a* + *4b*.
16. 19^{II} + *4a 4b*.

17–24. Progeny possible in backcross of monosomic F_1 ♀ × Thatcher to develop chromosome-deficient lines.
17. 21^{II}.
18. 19^{II} + ch. 3 as in F_1.
19. 19^{II} + ch. 4; dup. 4*a*, def. 10*a*.
20. 20^{II} + 1^{I}.
21. 19^{II} + ch. 4; dup. 4*b*, def. 10*b*.
22. 20^{II} + 1^{I}.
23. 19^{II} + ch. 5; dup. 4*a* + 4*b*.
24. 19^{II} + 2^{I}.

* *Deficiencies and duplications throughout this figure refer not actually to numbers of chromosomes, but to normal gene content.*

to be expected. These are of interest, for they represent two different whole chromosome deficiencies of the Thatcher karyotype. The type illustrated at 12 would be deficient for 4*a* ▬▭ 10*b* and that at 14 for 10*a* ▭▬ 4*b*. If two such lines are developed and used, complications arising from the structural changes can be eliminated (cf. (7)). The worker must, of course, be in a position to ascertain whether any two monosomics so derived are deficient for different chromosomes. This can be done by intercrossing them. If they are the same, the 40-chromosome progeny will have 20^{II} at meiosis; if different, there will be 19^{II} 2^{I} at meiosis, that is, the 40-chromosome progeny will not be truly nullisomic, but doubly monosomic.

Translocations are not the only complications that may be encountered, however. Person (7) has reported that a fairly high incidence of asynapsis occurs at meiosis of F_1 monosomics. This may result in what he terms "univalent shift", that is, progeny from such monosomics may be obtained that might possibly be deficient for a chromosome other than the one for which the parent was deficient. In practice, it must be apparent that all derived lines should be checked against the standard, possibly during the process of development, but certainly when the program is completed.

2. Transfer of Single Chromosomes from Donor Varieties to Chromosome-deficient Lines to Develop Substitution Lines

When chromosome-deficient lines have been developed in a desired variety, chromosomes from other varieties can be transferred to develop substitution lines. For purposes of illustration, three methods for the transfer of chromosome X from Timstein to Thatcher will be described:

(*a*) *The Use of a Nullisomic as the Recurrent Parent*

Step 1. Nulli-X of Thatcher ♀ is crossed with Timstein ♂.

Step 2. The F_1 are all monosomic; the univalent being chromosome X of Timstein. This F_1 is used as the male parent on nulli-X of Thatcher ♀.

Step 3. The backcross 1 progeny is of two types. Approximately 94–99% of the plants are monosomic; the univalent again being chromosome X of Timstein, while 1–6% are nullisomic. The monosomic backcross derivatives are repeatedly backcrossed as the pollen parents to nulli-X of Thatcher until the genotype of Thatcher for the 20 chromosomes other than chromosome X is sufficiently reconstituted. After the last backcross has been made, the monosomic progeny are permitted to self. Approximately 24% of the progeny after selfing will be disomic for chromosome X of Timstein. These plants will in fact represent the substitution line, which may be designated "Thatcher (2X Timstein)".

In a like manner substitution lines of other chromosomes from the desired donor varieties can be produced.

(*b*) *The Use of a Line as the Recurrent Parent Having an Isochromosome or a Telocentric of the Critical Chromosome as the Recurrent Parent*

Because lack of fertility or vigor may frequently make it impracticable to use nullisomics of the recipient variety, lines monosomic for either telocentrics

TABLE I

DESCRIPTION OF GAMETES AND PROGENY OF CROSSES INVOLVING ISO-X LINE THATCHER ♀ × TIMSTEIN ♂, AND ISO-X LINE THATCHER ♀ × MONOSOMIC F_1 OR BACKCROSS

Female parent \ Male parents: Eggs / Pollen	Timstein	Monosomic F_1 or backcross	
	21^{I} (100%)	21^{I} (96% approx.)	20^{I} (4% approx.)
Iso-X Thatcher — 20^{I} (75% approx.)*	$20^{II} + 1^{I}$ (X Tim.) (75% approx.)	$20^{II} + 1^{I}$ (X Tim.) (72% approx.)	20^{II} (3% approx.)
Iso-X Thatcher — 20^{I} + iso-X (25% approx.)	20^{II} + iso-X + normal X (25% approx.)	20^{II} + iso-X + normal X (24% approx.)	2^{II} + iso-X (1% approx.)

**These figures are probability calculations, based on the frequencies reported by Sears (10). Experience has shown that the proportions obtained vary with the chromosome involved and also with the environment.*

or isochromosomes of the deficient chromosome can be used to advantage. The essential features of the procedure involved and the different types of gametes and progeny produced when isochromosomes are used are described in Table I. It is assumed that chromosome X of Timstein will be transferred to Thatcher and that the recipient aneuploid line has an isochromosome X of Thatcher. Frequencies of the different types of functional gametes as reported by Sears (10) have been used in making the calculations. As can be seen from the table, this method is as rapid as the one using nullisomics as recurrent female parents. Since in every generation over two-thirds of the plants have as the univalent the chromosome to be transferred, relatively few plants need to be examined in each backcross generation. These monosomics are used as the recurrent male parents in each successive backcross until the genotype of Thatcher has been sufficiently reconstituted. Selfing of the final backcross monosomics will again result in plants that have a pair of substituted chromosomes X of Timstein.

(*c*) *The Use of Monosomics as Recurring Female Parents*

Ordinary monosomics also can be used in the production of substituted lines. However, to avoid the possibility of obtaining monosomic offspring that derive their univalent from the female, that is the recipient variety, instead of the male, that is the donor variety, one generation of selfing must precede each further backcross.

Briefly the procedure is as follows:

Step 1. Monosomic X of Thatcher is crossed with Timstein.

Step 2. The F_1 is analyzed cytologically, and monosomic F_1's are selected and allowed to self. These monosomic F_1's derive their univalent from Timstein.

Step 3. In the F_2 (obtained from selfing monosomic F_1's) plants with 21^{II} are used as pollen parents on monosomic X of Thatcher since these plants will have a pair of chromosomes X of Timstein.

Steps 4–*n*. Again the backcross is cytologically studied; monosomics are selected and selfed and their disomic progeny used as pollen parents to monosomic X of Thatcher. This process, of crossing the pollen plants that are disomic for chromosome X of Timstein to monosomic X of Thatcher, is repeated until the genotype for Thatcher represented by the other 20 chromosomes has been sufficiently reconstituted. The final backcross plants with 20^{II} 1^{I} when selfed will produce some disomics with a pair of chromosomes X from Timstein, i.e., they will again be "Thatcher (2X Timstein)".

The disadvantages of using monosomics are obvious. Twice as many generations of plants must be grown and many more cytological examinations are necessary because of the need to self after every backcross. On the other hand, lines with telocentrics or isochromosomes have not yet been obtained in most varieties in which substituted lines are being developed. Under these conditions monosomics must be used and the outlined procedure should be followed, unless nullisomics are available, or the chromosome to be transferred from the donor variety carries clearly recognizable marker genes.

3. The Transfer of a Chromosome Involved in a Reciprocal Translocation

Sears (11) has outlined a procedure of transferring a chromosome involved in a reciprocal translocation. His procedure is based on the assumption, however, that the chromosome to be transferred will always be associated with the two chromosomes from the recipient variety as a chain of three at metaphase 1 and that alternate disjunction will prevail at anaphase 1. This would be the ideal situation for the worker on monosomics. Frequently, however, he will be working with reciprocal translocations that involve only small segments, or in which crossing over in the translocated segments fails; in any event, the three chromosomes of a monosomic F_1 or backcross will not regularly be present as a chain association of three at metaphase 1 of meiosis.

If the three chromosomes persist regularly as a chain of three to metaphase 1 of meiosis, and if disjunction is alternate, the monosomic F_1 and successive backcrosses obtained from crossing to nullisomics of the recipient variety will have the karyotype illustrated at 9 in Fig. 1. There are, however, alternative possibilities if the above conditions do not always prevail.

The gametes that may be functional and the progeny produced when they combine with the gametes of the recipient variety are again shown in Fig. 1. Of special interest to the breeder are the karyotypes shown at 11 and 13 in the figure. It is obvious that these "monosomics" would also be able to form 20^{II} 1^{I} or 19^{II} c^{III} at metaphase 1 of meiosis. But these are not monosomics in the true sense of the term. They are in fact "deficiency duplication monosomics" since each is deficient for only part of chromosome X of Thatcher and duplicated for part of chromosome IV of Chinese Spring in this case. Obviously, of the three monosomic types, only that shown at 9 is of

any use to the breeder. The question is, how to distinguish between 9, 11, and 13. The only method of differentiation would be relative frequency of chain formation. In 11 and 13 there are two chromosomes in the complex that are homologous for their entire length. This likely will result in complete pairing of these two chromosomes in which case a chain of three chomosomes will rarely form in plants with such karyotypes. The breeder then must avoid using in his backcrossing program plants in which chains of three are rarely or never observed and must select plants with a chain-of-three frequency similar to that observed in the monosomic F_1.

4. Alien Substitutions

Alien substitutions involve the transfer of chromosomes from other species or even genera to chromosome-deficient lines of wheat. In spite of the detrimental effects conferred by parts of alien chromosomes, it is, nevertheless, conceivable that certain substitutions may be beneficial. O'Mara (6) has shown that replacement of chromosome IX with one of the chromosomes of rye resulted in plants with near normal fertility and vigor whereas the nulli-IX in wheat has low fertility and lacks vigor. The same chromosome present as an addition to the normal complement, on the other hand, results in semidwarf, partially female-sterile plants. It may be that the restoration of the number to 42 is important from the standpoint of balance; in any event it is clear that the effect of an alien chromosome substitution cannot be predicted from what is observed when the chromosome is present as an addition.

To illustrate one method of alien chromosome substitution the procedure used by Jenkins (2) for the development of wheat lines having specific chromosomes replaced by rye chromosomes will be briefly described. Sears (12) and Hyde (1) have used a different method involving a bridging cross to obtain addition lines of *Haynaldia villosa* chromosomes.

Step 1. Wheat is crossed with rye to produce a sterile F_1.

Step 2. The F_1 is doubled to produce a fertile amphiploid.

Step 3. The amphiploid is crossed to common wheat. This produces an F_1 having 21 pairs of wheat chromosomes and seven univalent rye chromosomes. Selfing such an F_1 or backcrossing to wheat will result in elimination of most of the rye chromosomes and in the production of alien addition lines monosomic or disomic for the different rye chromosomes.

Step 4. Alien substitution lines can now be produced by crossing any one or all of the nullisomics in wheat ♀ with the alien addition lines ♂. The F_1 of such a cross will have 42 chromosomes, and will be of the constitution $20^{II} + 2^{I}$. It will have one univalent from wheat and one univalent corresponding to the rye chromosome in the alien addition line. On selfing such a plant two types of progeny with 21 pairs at meiosis may be produced. One type will be a normal wheat line; the other the alien substitution line. Should these two types be indistinguishable morphologically, it is a simple matter to determine which are normal and which are alien substitution plants by crossing them with wheat. Only plants with the substitutions will result in hybrids incapable of forming 21^{II} at meiosis I.

5. Combination of Two Chromosome Substitutions into a Single Line

When desirable genes have been associated with different chromosomes of different varieties, and when such chromosomes from different varieties have been transferred singly into a recipient variety, it may be desired to bring the two chromosomes together into a single line. Thus, it is now known that chromosome X of Timstein carries two different genes for stem rust resistance, and that the McMurachy gene for 15B rust resistance is carried by chromosome XX. One phase of the aneuploid program at the University of Alberta is to develop two Thatcher substitution lines, one with chromosome X of Timstein, the other with chromosome XX of McMurachy. When these have been established it is planned to combine these into a single line of Thatcher having both chromosome X of Timstein and chromosome XX of McMurachy.

Two different procedures can be used in combining these substitution chromosomes into a single line. Both will be briefly outlined.

Procedure (*a*)

In this procedure, one of the substitution lines is made deficient for the critical chromosome of the other substitution by the method outlined earlier. Thus, one might use Thatcher (2X Timstein) as the recurring male parent and cross to nulli-XX of Thatcher. The monosomic F_1 of this cross and later backcross derivatives would be used as the female parent in backcrosses to line Thatcher (2X Timstein) until Timstein X had been sufficiently reconstituted. The final monosomic derivative on selfing would provide a few nullisomic plants. These plants, in addition to being nullisomic for chromosome XX, will be homozygous, or nearly so, for the factors carried by chromosome X of Timstein.

Substitution line Thatcher (2XX McMurachy) is then crossed to such a nullisomic, and monosomic F_1 and backcross derivatives are repeatedly backcrossed to this nullisomic line until chromosome X of Timstein has been sufficiently reconstituted. After selfing, the final monosomic backcross derivative will produce some plants that are disomic for chromosome XX of McMurachy and chromosome X of Timstein, which has been reconstituted twice through backcrossing. The disadvantages of this method are (*a*) the time and work involved; it would take six to eight generations in each phase of the above program or a total of 12 to 16 before double substitution would be complete and (*b*) the opportunity in both phases of the program of crossing over between chromosome X of Timstein and that of Thatcher. This introduces the possibility that some genes of this chromosome from Timstein will not be present on the reconstituted substitution chromosome. They may be important.

Procedure (*b*)

This procedure will also deal with combining chromosome X of Timstein and XX of McMurachy into a single line.

Step 1. Nulli-X is crossed with nulli-XX of Thatcher. The progeny will be plants of Thatcher that are doubly monosomic, that is deficient for one each of chromosomes X and XX.

Step 2. Using this doubly-monosomic line as the female, crosses are made with each of the lines Thatcher (2X Timstein) and Thatcher (2XX McMurachy).

In Table II are given different types of progeny that can be expected from these crosses. Since the frequencies with which different types of gametes are produced and function are not known, no attempt is made to calculate the proportions of the different types of progeny. The important point is that female gametes with 19 chromosomes have been found to be functional in producing doubly-monosomic progeny (7). One group, derived from the cross with Thatcher X (Timstein) is monosomic for X Timstein and XX Thatcher, the other for X Thatcher and XX McMurachy.

Step 3. Doubly-monosomic plants from these two groups are crossed with each other.

TABLE II

GAMETES AND PROGENY PRODUCED IN THE CROSS BETWEEN THATCHER MONOSOMIC FOR X AND XX AND EACH OF THE SUBSTITUTION LINES THATCHER (X TIMSTEIN) AND THATCHER (XX MCMURACHY)

Female parent / Male parent: Gametes		Thatcher X (Tim.)	Thatcher XX (McM.)
		All gametes 21 chromosome including X Timstein	All gametes 21 chromosome including XX McMurachy
Doubly monosomic deficient for X That. and XX That.	21 chromosomes	[1] 21^{II} X Tim. X That.	[5] 21^{II} XX McM. XX That.
	20 chromosomes def. for XX That.	[2] 20^{II} 1^{I} (def. XX That.)	[6] 20^{II} + 1^{I} (def. XX McM.)
	20 chromosomes def. for X That.	[3] 20^{II} 1^{I} (def. X Tim.)	[7] 20^{II} 1^{I} (def. X That.)
	19 chromosomes	[4] Doubly monosomic 19^{II} + X Tim. + XX That.	[8] Doubly monosomic 19^{II} + X That. + XX McM.

In Table III are listed the progeny that might be expected when two double monosomics are crossed. It is impossible with any justification to list expected frequencies, because, as has been pointed out earlier, data are lacking to permit making probability frequencies since the frequency of elimination of the univalent is different for different chromosomes and for different conditions.

Three of the gametic combinations can result in offspring with 19^{II} and 2^{I}, as shown at 6, 8, and 10 in Table III. Of these, only that shown at 6, with X of Timstein and XX of McMurachy as the two univalents, is capable of producing the desired offspring with the double substitution, that is, plants

TABLE III

PROBABLE KINDS OF GAMETES AND PROGENY PRODUCED IN CROSSING TWO DOUBLE MONOSOMICS

Female parent	Types of gametes	Male parent: Doubly monosomic, 19^{II} + X Tim. + XX That. — Types of gametes: 21 chromosomes X Tim. XX That.	20 chromosomes def. for X Tim.	20 chromosomes def. for XX That.
Doubly monosomic 19^{II} + X That. + XX McM	21 chromosomes X That. XX McM.	[1] 21^{II} X That. X Tim. XX That. XX McM.	[2] Monosomic. 19^{II} + XX That. + XX McM. + X That.	[3] Monosomic. 19^{II} + X That. + X Tim. + XX McM.
	20 chromosomes def. X That.	[4] Monosomic. 19^{II} + XX That. + XX McM. + X Tim.	[5] Nullisomic. 19^{II} + XX That. + XX McM.	[6] Doubly monosomic. 19^{II} + X Tim. + XX McM.
	20 chromosomes def. XX McM.	[7] Monosomic. 19^{II} + X That. + X Tim. + XX That.	[8] Doubly monosomic 19^{II} + X That. + XX That.	[9] Nullisomic. 19^{II} + X That. + X Tim.
	19 chromosomes def. X That. and XX McM.	[10] Doubly monosomic 19^{II} + X Tim. + XX That.	[11] 19^{II} 1^{I} XX That. Nulli-X	[12] 19^{II} 1^{I} X Tim. Nulli-XX

with 19 pairs of chromosomes from Thatcher, a pair of chromosomes X from Timstein, and one pair of chromosomes XX from McMurachy. In this particular instance combination 6 can be readily distinguished from combinations 8 and 10 because it is the only double monosomic carrying chromosome XX from McMurachy. This chromosome carries the gene for spike compactness, and plants of constitution 6 will have semicompact spikes.

Discussion

Sears (10) has already demonstrated the value of substitutions in locating, and especially in determining the mode of action of certain genes. Work at the University of Alberta during the past few years (3) has indicated rather clear-cut effects of certain substituted chromosomes on such characters as lodging, height, earliness, yield, etc. Thus it is certain that the genetic effects of individual chromosomes on these important characteristics can be more or less intensively studied. Information on the genetics of these characters will be of great value in planning future breeding work.

Work at the University of Alberta (3) has demonstrated again the well-known fact that characters such as yield, height, and earliness in wheat are often governed by two or more interacting gene pairs. With substitution lines, where genetic effects of individual chromosomes can be studied, it is possible under appropriate conditions to isolate certain gene pairs from the

system to which they normally belong and to study their individual actions. Disease resistance may be taken as an example. For the varieties Timstein, McMurachy, and Thatcher mentioned earlier in this study, the reactions to a large number of races is known. It will be interesting and valuable to determine next the reaction when a chromosome carrying a gene or genes giving resistance to different races is introduced to replace a chromosome in the recipient variety. Thus, when chromosome XX of McMurachy is introduced to replace chromsome XX of Thatcher, one can determine (*a*) the reaction of the line having a single dose (monosomic plants) of the gene(s) on this chromosome, and (*b*) the effect when the dosage is doubled as it is in a substitution line Thatcher (2XX McMurachy). The important thing is whether this chromosome of McMurachy, now present in a new genetic system, will confer resistance to the same rust races as it did before it was moved to Thatcher. While there is some evidence to indicate that major effects such as speltoidy controlled by the *Q* factor on chromosome IX (MacKey (5)) are more or less autonomous in producing their effects, it is very likely that characters influenced by minor as well as major genes and their interactions will be influenced in such a way that a prediction of the result of a substitution would be impossible. In any case, gene interaction can be more precisely studied when the dosage of genes on at least one of the chromosomes involved can be more completely controlled. Results obtained at this institution (3) have shown very striking effects on quantitative characters such as yield, resistance to lodging, earliness, etc., from chromosome substitutions involving Chinese Spring as the recipient variety. Such results indicate that at least the genes associated with certain chromosomes have fairly autonomous action so far as producing certain morphological effects are concerned. It remains to be determined in future studies how extensive this phenomenon is and under what conditions it fails.

With further analyses a greater number of useful genes, and the chromosomes which carry them, will be known. It is probable that the method of chromosome substitution, exacting though it is, will be more generally used in moving these known genes from one variety to another. The method of chromosome substitution thus has application in both the theoretical and practical fields. It offers definite possibilities of success, but, like all breeding methods, it has drawbacks and limitations.

References

1. HYDE, B. B. Addition of individual *Haynaldia villosa* chromosomes to hexaploid wheat. Am. J. Botany, 40 : 174-182. 1953.
2. JENKINS, B. C. Substitutions of rye chromosomes in winter wheat. Proc. Sixth Meeting of Assoc. Comm. Plant Breeding, Y-2. Feb., 1951.
3. KUSPIRA, J. Cytogenetic and genetic analyses of certain characters in common wheat using whole chromosome substitution lines. Ph.D. Thesis, University of Alberta Edmonton, Alta. April, 1955.
4. LARSON, R. I. Aneuploid analysis of inheritance of solid stem in common wheat. Genetics, 37 : 597-598. 1952.
5. MACKEY, J. Neutron and X-ray experiments in wheat and a revision of the speltoid problem. Hereditas, XL : 65-180. 1954.

6. O'MARA, J. G. The substitution of a specific *Secale cereale* chromosome for a specific *Triticum vulgare* chromosome. Records Genetics Soc. Amer. 15 : 62-63. 1946.
7. PERSON, C. Some aspects of monosomic wheat breeding. Can. J. Botany, 34 : 60-70. 1956.
8. SEARS, E. R. Cytogenetic studies with polyploid species of wheat. I. Chromosomal aberrations in the progeny of a haploid of *Triticum vulgare*. Genetics, 24 : 509-523. 1939.
9. SEARS, E. R. Cytogenetic studies with polyploid species of wheat. II. Additional chromosomal aberrations in *Triticum vulgare*. Genetics, 29 : 232-246. 1944.
10. SEARS, E. R. Nullisomic analysis in common wheat. Am. Naturalist, 87 : 245-252. 1953.
11. SEARS, E. R. Addition of the genome of *Haynaldia villosa* to *Triticum aestivum*. Am. J. Botany, 40 : 168-174. 1953.
12. UNRAU, J. The use of monosomes and nullisomes in cytogenetic studies of common wheat. Sci. Agr. 30 : 66-89. 1950.

CYTOLOGICAL STUDIES OF ASCUS DEVELOPMENT IN COCHLIOBOLUS SATIVUS[1]

By S. B. Hrushovetz[2]

Abstract

Conjugate divisions occurred in the binucleate crozier of *Cochliobolus sativus* (Ito & Kurib. ex Kurib.) Drechs. ex Dast. Two cross walls were laid down in such a way that the penultimate cell was binucleate. A lateral bulge developed in this cell and it quickly assumed the clavate shape of the young ascus. The two nuclei in the clavate ascus fused and this was accompanied by a fusion of their nucleoli. In meiosis two divisions occurred with pairing between highly contracted chromosomes early in the first division. A third division (mitotic) followed and resulted in the formation of eight haploid nuclei. Ascospore delimitation began at this stage and each spore was initially uninucleate. Evidence of structural hybridity was seen in all divisions in the ascus. The haploid chromosome complement, as determined from pachytene and metaphase stages, consisted of seven or eight chromosomes.

Introduction

Cochliobolus sativus, the perfect or ascigerous stage of *Helminthosporium sativum*, can be readily obtained in culture. It was first described by Kuribayashi (3). Tinline (10), who found the fungus heterothallic, made a detailed study of factors affecting perithecial formation and maturation. He attributed the occurrence of asci with fewer than eight ascospores to the inhibitory effect of light on ascospore delimitation (11). Kuribayashi also had reported that mature asci often contained fewer than eight ascospores. Shoemaker (8), however, found that the pairing of various isolates with strains 104*c* and 104*d* never resulted in the production of mature asci, though young perithecia with numerous immature asci were always formed. It is apparent that light was not the only factor inhibitory to ascospore delimitation and it is possible that in certain matings structural hybridity of chromosome complements prevents normal meiotic and mitotic division.

Although the prime purpose of this study was to determine the origin of the nuclei in the multinucleate, multicellular ascospore, it was felt that a study of the chromosomal behavior in the developing ascus might offer some explanation for Tinline's observation that mature asci frequently contained abnormal ascospores and that asci with fewer than eight ascospores were often found.

Materials and Methods

Monoascosporic isolates of a fertile mating pair of *Cochliobolus sativus*, designated as T_2 (*A*) and A_{18} (*a*), were obtained from Tinline, and the present study was made exclusively on this mating pair. The cultural method used

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for the production of perithecia was that outlined by Tinline (11). Preliminary studies showed that if barley seeds containing young perithecia were transferred to fresh Sachs' agar medium, new perithecia developed on the agar at some distance from the seeds (1). In these new perithecia the asci were all approximately at the same stage of development.

The author tried the Feulgen squash technique as outlined by McIntosh (5), with and without aceto- or propiono-carmine, but found it inadequate for detailed chromosome studies. Several synthetic and natural carmines and orceins were also tested and abandoned. One natural orcein, Michrome, secured from Edward Gurr Ltd., London, England, gave promising results. The Giemsa stain (1, 2) appeared to be best for staining chromosomes. A limited number of acetocarmine and propionocarmine mounts were useful for a study of the nucleolus.

Blocks of agar containing the perithecia of *C. sativus* were placed in a Carnoy's type (9) fixative (six parts absolute ethyl alcohol, one part glacial acetic acid, one part lactic acid) for 10 min., rinsed in 95% ethyl alcohol, and then transferred to 70% ethyl alcohol. If such material is stored in the refrigerator for several weeks the staining of the achromatic figure is often enhanced. The perithecia were then freed from the substrate, punctured with a needle to facilitate subsequent penetration of reagents, placed in cold 1 *N* HCl for 10 min., hydrolyzed in 1 *N* HCl at 60° C. for seven minutes, washed several times in distilled water, and finally placed in phosphate buffer solution (2) of pH 6.9. The asci and the stromatic contents of the perithecia (pseudothecia) were carefully removed, freed from the perithecial wall, and placed in fresh buffer in a small vial. Since the Giemsa stain penetrates tissues rather slowly, an equal portion of the Giemsa stain, as prepared by British Drug Houses, was added to the buffer, and the material was usually left in the staining solution overnight. The vial containing the staining solution was stoppered to prevent excessive coagulation of the stain resulting from evaporation of the methyl alcohol. The stained perithecial contents were placed on a culture slide with a depression 2 mm. deep. They were moved, two or three at a time, to a drop of the buffer solution, which removed any adhering coagulated stain, and then they were transferred to a microscope slide containing a drop of buffer. A cover glass was applied and the stained material was carefully spread by pressure exerted with a dissecting needle. While excess buffer was being removed with filter paper, gentle pressure was applied to the cover glass by the thumb or forefinger to prevent curling of the asci. The slides were ringed with Abopon, a colorless water-soluble mounting medium obtained from Glyco Products Co. Inc., 26 Court Street, Brooklyn 2, New York. Attempts to make HCl-Giemsa-stained material permanent with Euparal or Canada balsam were unsuccessful.

Preliminary studies showed that the morphology of the chromosomes was best studied before the bivalents reached their maximum length. Size was the main criterion of chromosome differentiation, since chromomeric regions were not conspicuous. Chromosomes were designated by number in order of

decreasing length, a scheme patterned after that of McClintock (4) and Singleton (9) for *Neurospora*. When chromosomes were of approximately equal length other features were used for differentiation. The characterization of the specific chromosomes is tentative and is based on 15 well-spread preparations representing early to mid pachytene stages of one type of mating, namely T_2 (*A*) $\times$ A_{18} (*a*).

The terminology applied to the various division stages is that outlined by Singleton (9). Thus metaphase I is the metaphase of the first meiotic division, and anaphase III the anaphase of the third division. Interphase I is the interphase between the first and second meiotic divisions.

Observations and photographs were made on a standard Leitz Wetzlar microscope, fitted with a Leitz Wetzlar 1/12 fluorite oil immersion objective (95×, N.A. 1.32) and an oil immersion aplanatic achromatic condenser (N.A. 1.40). The illumination, rheostat-controlled, was provided by a 300-watt ribbon filament bulb fitted in an American Optical assembly and provided with a yellow-green Wratten filter. The photomicrographs were taken with a Leica 35-mm. camera containing the necessary microscopic attachments, on Ilford Micro Neg film, developed in Microdol, and printed on Kodabromide F4 or F5 paper.

Results

A summary description of the various nuclear divisional stages in the developing ascus is first presented so that a coherent picture of 'ascosporogenesis' may be obtained. This is followed by a detailed description of the processes involved.

Simultaneous mitosis of the two nuclei in the binucleate crozier occurred. Two cross walls were then laid down forming three cells: a terminal uninucleate cell, a penultimate cell containing two nonsister nuclei, and a basal uninucleate cell. A lateral bulge developed in the penultimate cell and it gradually assumed the clavate shape of the developing ascus. The two nuclei usually fused before much enlargement of the ascus occurred. This was soon followed by a fusion of their nucleoli. Synapsis of the much contracted chromosomes now began, accompanied by an enlargement of the ascus and its fusion nucleolus. Meiosis consisted of two divisions, Division I and Division II, and was followed by a mitotic division, Division III, which resulted in eight free haploid nuclei. Ascospore delimitation began at the eight-nucleate stage, there being generally another mitotic division, Division IV, before the developing ascospores had come to occupy the entire length of the ascus. Finally, several mitotic divisions accompanied by the formation of transverse septa produced eight multinucleate, multiseptate ascospores which enlarged and became coiled in a helix.

Data in Table I show that asci may reach their full length in pachytene so that ascus length cannot be used as a criterion for seriation of late meiotic stages. However, length of the ascus is useful in arranging in order the early prophase stages of Division I, which were sometimes difficult to interpret.

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TABLE I

COMPARISON OF LENGTH OF ASCUS AND STAGE OF NUCLEAR DEVELOPMENT IN *Cochliobolus sativus* AS DETERMINED BY SQUASH PREPARATIONS

Nuclear stage	Nuclei examined	Average ascus length (μ)	Range of ascus length (μ)
Presynapsis	6	23	14– 30
Postsynaptic to mid pachytene	39	85	33–111
Late pachytene	4	167	150–180
Metaphase to end of Division I	6	153	95–210
Division II	10	162	95–175
Division III	6	192	95–263

Stages Prior to Synapsis

The ascogenous hyphae were easily differentiated from the stromatic tissue by their broader, shorter cells and more deeply staining nuclei. They were generally binucleate, although some multinucleate cells were also observed. The terminal cell of an ascogenous hypha bent into a hooklike shape or crozier. The two nuclei, each with a well-defined nucleolus (1.0–1.5 μ in diameter) then entered the neck portion of the crozier, where they remained and began a simultaneous mitosis (Fig. 1). The chromosomes appeared greatly extended and definite chromomeric regions were visible on them. Contraction of the chromosomes then occurred (Fig. 2), and this was followed by metaphase and anaphase stages which resulted in the formation of four nuclei (Fig. 3). Tentative counts of late prophase stages suggested that seven or eight chromosomes were present in these haploid nuclei. Spindles were not observed even following the 'storage and hydrolysis' pretreatment suggested by Singleton (9). Two transverse septa were laid down resulting in the formation of three cells, the middle or penultimate cell being binucleate (Fig. 4). Nuclear fusion did not occur at this stage even though the two nuclei lay quite close together. The terminal cell sometimes increased in length and fused with the basal cell. Either this fusion cell or the penultimate cell could develop a new hook and conjugate mitosis was then repeated.

All magnifications are × 1800 unless otherwise stated and refer to Giemsa-stained material from the T_2 (*A*) × A_{18} (*a*) mating.

FIG. 1. Early prophase stage of conjugate division in crozier. × 3600.

FIG. 2. Prometaphase of conjugate division in crozier. × 3600.

FIG. 3. Telophase of crozier division. × 3600.

FIG. 4. Cross wall formation in crozier. Note binucleate penultimate cell. × 3600.

FIG. 5. Eight contracted chromosome bivalents in the fusion nucleus of a young ascus.

FIG. 6. Approximately 16 unsynapsed contracted chromosomes in a young ascus. × 3600.

FIGS. 7–11. Early to mid pachytene.

FIG. 8. Camera lucida drawing of the ascus in Fig. 7. Chromosome 4 bivalent is superimposed on the chromosome 2 bivalent.

FIGS. 9 and 11. The symmetrical cross shown in the lower left portion of the nucleus in Fig. 9, and in the central portion of the nucleus in Fig. 11 is the chromosome 4 bivalent.

FIG 12. Late pachytene.

FIGS. 13 and 14. Early diplotene.

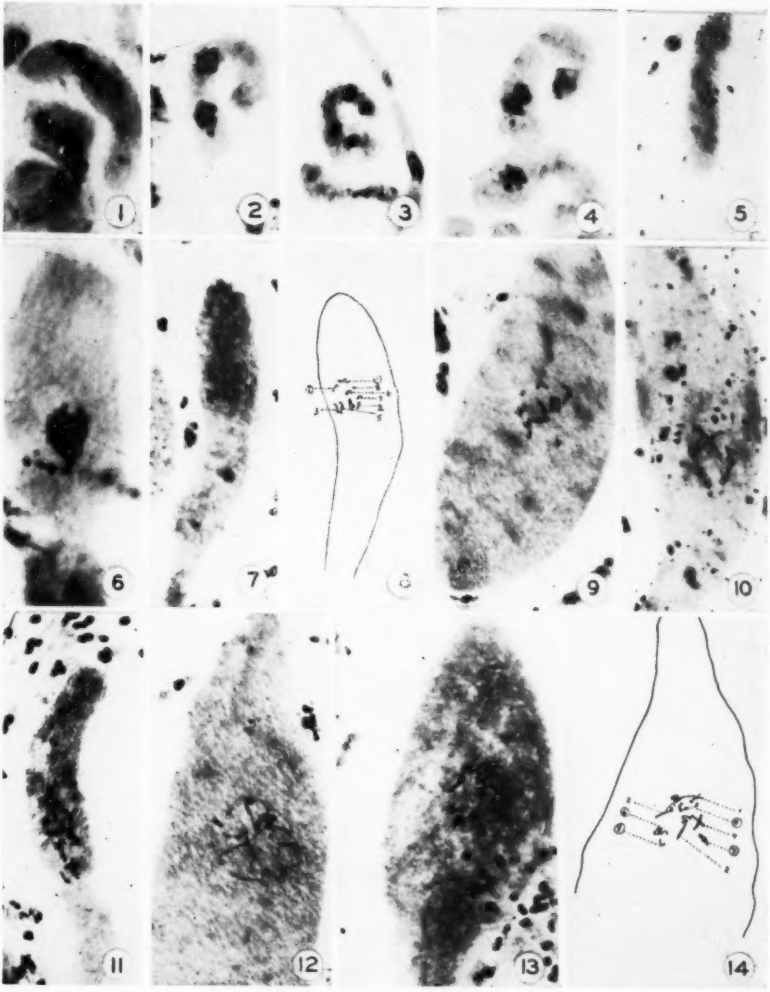
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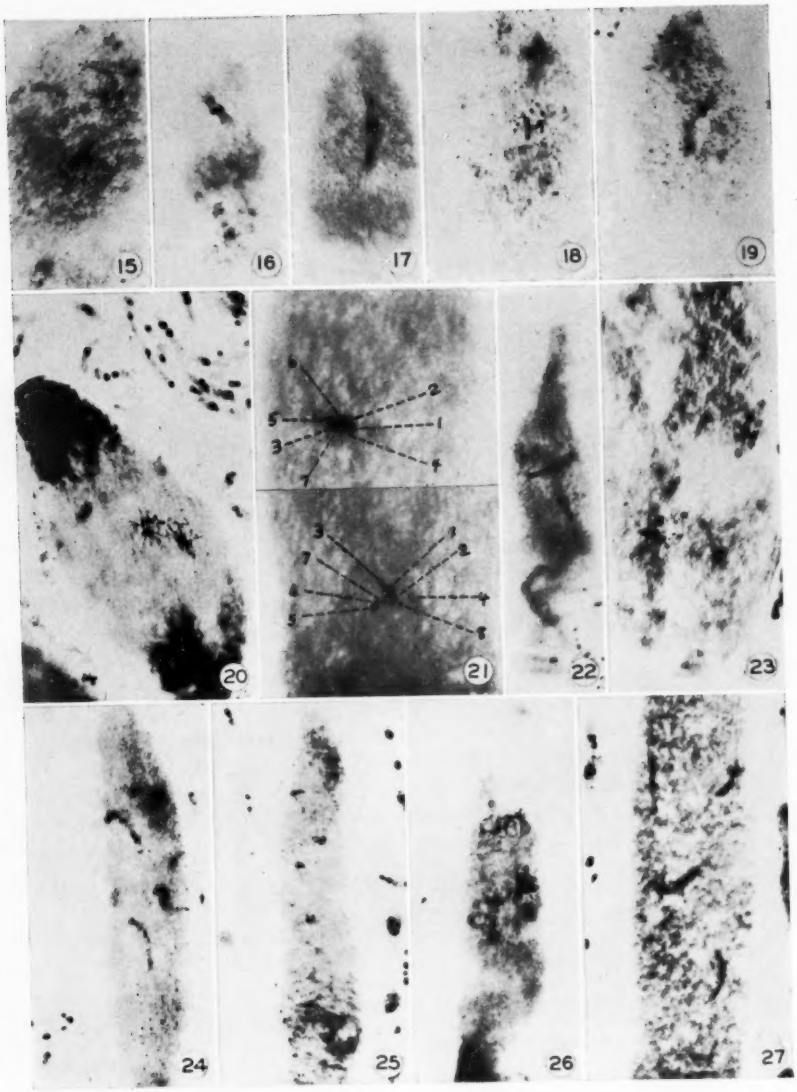
PLATE II



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On the side of the penultimate cell appeared an outgrowth which quickly assumed a clavate shape and subsequently became a young ascus. The two nuclei entered the ascus and fused. This was soon followed by a fusion of their nucleoli. The chromosomes of the two nuclei were highly contracted at this stage and either remained as two separate masses or were randomly distributed in the fusion nucleus (Fig. 6).

Synapsis to Late Pachytene

Synapsis is defined as the pairing of homologous chromosomes, and late pachytene as the stage of maximum extension of the paired chromosomes or bivalents. Fig. 5 shows a young ascus with eight synapsed bivalents. The size of the ascus and the length of the bivalents strongly suggest that synapsis must have occurred between highly contracted chromosomes. After synapsis the ascus enlarged and the bivalents underwent extension (Figs. 7, 8, 10). Increase in size of the fusion nucleolus also kept pace with that of the ascus. Chromomeres became very prominent when the bivalents reached their maximum extension (Fig. 12), and at this stage some of the bivalents measured over 20 μ in length.

Early to mid pachytene figures (Figs. 7, 9, 10, 11) indicated a minimum of eight bivalents, two very long ones of approximately equal length and measuring over 16 μ, three of medium length measuring 6–8 μ, and three short bivalents measuring less than 5 μ. Although homologous bivalents in different asci are expected to assume a variety of forms the author feels convinced that within the three size classes individual chromosomes may be recognized by consistent configurational characteristics as follows: a "T" or cross configuration (Number 1; Figs. 7, 8, 13, 14); two achromatic regions located approximately 4.5 μ from either end (Number 2; Figs. 7, 8); a diverging end (Number 3; Figs. 13, 14); a symmetrical cross or if poorly flattened a broad rod (Number 4; Figs. 13, 14); a straight rod more than 5 μ in length (Number 5; Fig. 7); short straight rods 3–4 μ in length (Numbers

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All magnifications are × 1800 unless otherwise stated and refer to Giemsa-stained material from the T_2 (A) × A_{18} (a) mating.

FIG. 15. Mid diplotene. Note prominent chromatic and achromatic regions.

FIGS. 16 and 17. Metaphase I, the latter was obtained from the mating of T_2 (A) × 47; six of the seven bivalents are in focus. Note prominent astral fibers and rodlike centrioles.

FIG. 18. Early anaphase I. Approximately 16 univalents are present.

FIG. 19. Late anaphase I.

FIG. 20. Interphase I. Some of the long chromosome arms appear as double strands.

FIG. 21. Prometaphase II. In the upper nucleus six of the seven visible chromosomes are in focus. In the lower nucleus eight chromosomes are present.

FIG. 22. Metaphase II.

FIG. 23. Early anaphase II. Approximately 16 chromosomes are present in each nucleus.

FIG. 24. Late anaphase II. Note chromosomes dispersed along the entire length of the spindle.

FIG. 25. Telophase II. Note the flat heterochromatic region which corresponds to the 'beak' in *Neurospora*.

FIG. 26. Interphase II.

FIG. 27. Metaphase III. Note the elliptical spindles with dotlike centrioles. The lowest nucleus has a chromosome lying free of the spindle.

6 and 7; Figs. 7, 8); and a short straight rod less than 2 μ in length (Number 8; Figs. 7, 8, 9).

Maximum size of the nucleolus, 7.5 μ in diameter, was attained by late pachytene, after which it began to decrease in size. The nucleolus was not studied in the remainder of Division I or in the other divisions. Nucleoli were not generally visible in the stromatic tissue or the ascogenous hyphae, although they were frequently observed in Division IV in the ascospores where they measured 1.50–2.0 μ in diameter.

Late Pachytene to Metaphase II

Late pachytene, the stage of maximum chromosome extension, was followed by diplotene, or the period of chromosome contraction and condensation (Figs. 13, 15). Maximum contraction of the bivalents in Division I was reached at metaphase. The appearance in diplotene of large, deeply staining regions separated by achromatic portions (Fig. 15) made it difficult to obtain reliable chromosome counts at this stage, even though the bivalents were well separated. McIntosh (6) obtained similar diplotene figures in *Pyronema* using the Feulgen technique. At prometaphase I the eight bivalents were usually grouped quite closely with the two largest bivalents, measuring 2.5–3.0 μ in length. The other bivalents appeared as small spherical or rodlike bodies 1.0–1.5 μ. As the contraction of the bivalents proceeded, a broad truncated spindle with rodlike centrioles and prominent astral fibers was formed (Fig. 17). The bivalents then arranged themselves as a compact group at the equatorial region of the spindle which was orientated parallel or obliquely to the longitudinal axis of the ascus (Fig. 16). In early anaphase approximately 16 univalents or dyads were counted (Fig. 18), half of which passed to either pole. This polar movement was accompanied by spindle elongation (Fig. 19). Astral fibers were seen at metaphase I and anaphase I as well as at metaphase II and III. In telophase I the chromosomes stained very faintly with Giemsa, except at the very prominent heterochromatic regions. The chromosomes now underwent elongation and by interphase I chromosomes 1 and 2 were 7.5 μ or more in length (Fig. 20). Chromomeres

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All magnifications are × 1800 unless otherwise stated and refer to Giemsa-stained material from the T_2 (*A*) × A_{18} (*a*) mating.

FIGS. 28 and 29. Interphase III. Nuclei with variable amounts of chromatin material indicate that structural hybridity is present.

FIG. 30. Ascospore delimitation at the eight-nucleate stage. Note that each spore is initially uninucleate and that all nuclei are not at the same stage of division.

FIGS. 31 and 32. Synchronized anaphase IV in an ascus in which spore delimitation did not occur at the eight-nucleate state. Note free chromosome in nucleus 7 and trailing chromosomes in nuclei 2 and 5.

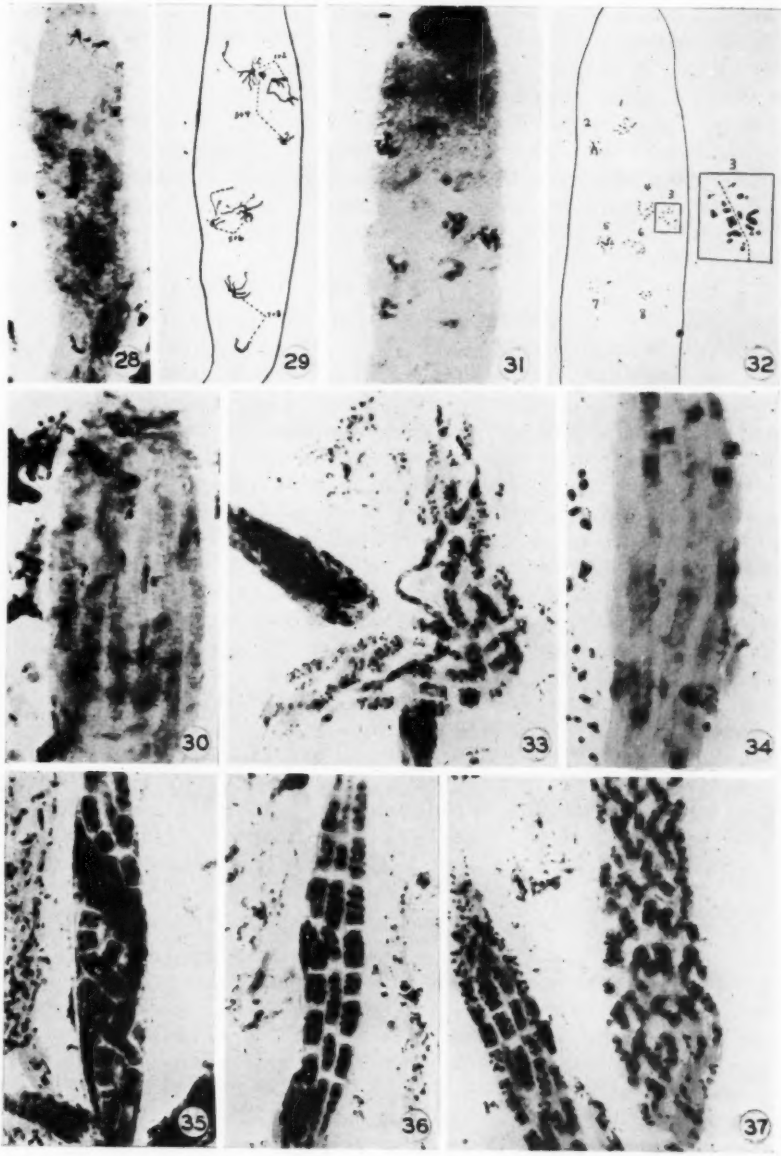
FIG. 33. Ascus containing six ascospores. Note degeneration of one of the ascospores. × 900.

FIG. 34. Normal ascus with eight binucleate ascospores. Note wide separation of the two nuclei in a spore, and the variation in timing of the stage of the nuclear division between and within spores.

FIG. 35. Ascus containing two normal ascospores. Note coiling of the spores. × 900.

FIG. 36. Ascus containing three ascospores. × 900.

FIG. 37. Asci containing four and eight normal ascospores. × 900.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became prominent and there was evidence of doubleness in some of the chromosome arms. The centromeres appeared to be aggregated in a small, deeply staining clump comparable to the 'beak' described in *Neurospora* by McClintock (4) and Singleton (9). The chromosomes now began to contract and at prometaphase II eight deeply staining chromosomes were present (Fig. 21). Differences in size of these dyads were sufficient to separate at least the two longer chromosomes, numbers 1 and 2, from the three short ones, numbers 6, 7, and 8. In a few cases only seven chromosomes were counted at this stage, although eight was the usual number (Fig. 21).

Metaphase II to Ascospore Delimitation

At metaphase II the eight dyads of the two nuclei took up positions at the equator of the spindle (Fig. 22). The spindles, which possessed prominent astral fibers and dotlike centrioles, varied with regard to their position and orientation in the ascus (Figs. 21, 22). They usually occurred in the upper part of the ascus and were orientated parallel to the longitudinal axis of the ascus, although sometimes they were present in the central or lower part and were orientated obliquely rather than parallel. Early anaphase II figures showed approximately 16 chromosomes in each nucleus (Fig. 23). In anaphase II the chromosomes after disjunction were dispersed along the length of the elongated spindle (Fig. 24). Polar movement of the contracted chromosomes and spindle elongation continued and at telophase II four well-separated almost spherical haploid nuclei with large chromocenters or heterochromatic regions were visible (Fig. 25). With telophase II, meiosis was completed. The chromosomes now began to elongate (Fig. 26), and the interphase II configurations (Fig. 26) closely resembled those of interphase I (Fig. 20). Third division stages closely resembled those of the second division. Metaphase III is shown in Fig. 27. Anaphase III and telophase III followed and resulted in the formation of eight nuclei (Figs. 28, 29). The haploid nuclei of Division III usually were distributed peripherally throughout most of the ascus in contrast to those of Divisions I and II where they were restricted to the central and upper part of the ascus.

Ascospore Delimitation and Further Divisions

Ascospore delimitation occurred at the eight-nucleate stage (Fig. 30); however the eight nuclei often entered Division IV without any evidence of ascospore formation. Under the latter conditions, nuclear divisions may or may not be synchronized. This appeared to depend on the position of the nuclei within the ascus. If the eight nuclei did not distribute themselves over the entire length of the ascus, then the fourth and later divisions were usually synchronized (Figs. 31, 32); if, on the other hand, the nuclei were well separated, then those in the upper part of the ascus were usually much further advanced in Division IV than were those nearer the base. In one ascus a gradation from metaphase IV to telophase IV was observed. In the metaphase figures of this ascus at least seven chromosomes were visible. The fate of such

asci that failed to delimit ascospores after the third division was not further investigated, but it appears doubtful that they ever do delimit normal spores.

In spore delimitation only a single nucleus was included in each ascospore initially (Fig. 30). The nucleus in each spore then entered Division IV and often before the ascospore had extended the full length of the ascus two separate nuclei were present (Fig. 34). The two nuclei in each spore then entered Division V. Both in Divisions IV and V extreme variability existed in the timing of the divisional stages within and between spores (Fig. 34). Several additional mitotic divisions followed and septa appeared at random in the spores. In normal mature asci, the eight ascospores were coiled in a helix (Fig. 37). Asci contained anywhere from one to eight apparently normal ascospores (Figs. 33, 35, 36, 37). Some asci contained degenerating ascospores (Fig. 33). Coiling of spores often occurred even if only two ascospores were present (Fig. 35). The number of cross walls in normal ascospores was as high as 14 and the number of nuclei as high as 30 per cell. The terminal cells of the ascospores usually had fewer than ten nuclei.

Discussion

It is apparent from the present study of *C. sativus* that spore delimitation begins at the eight-nucleate stage and that initially only one haploid nucleus is present in the delimited ascospore. Asci which contained more than eight free haploid nuclei were never observed to delimit ascospores. This, therefore, verifies the assumption made by Tinline (11) that the multinucleate–multiseptate ascospore is 'homogenous, barring mutation'.

Germination of an ascospore produces the imperfect stage known as *H. sativum*. It is obvious, therefore, that in a monoascosporic culture all the cells, including the multinucleate–multicellular conidia, are homokaryotic. Tinline (11) has shown from genetic studies that the ascospores are homokaryotic. He found that asci produced from matings of a pink and a dark strain always segregated for color and compatibility; the two loci involved were not linked. Pink and dark-colored conidia were never found occurring together in any monoascospore culture, nor did monoascospore cultures ever develop mature perithecia. Besides proving homokaryosis of the ascospores, these genetic studies also showed that the ascogenous hyphae, and particularly the young croziers, are heterokaryotic and include both types of nuclei. Therefore the simultaneous mitotic division which occurs in the crozier is a conjugate division.

In the present study meiotic stages were used for the determination of the haploid chromosome complement in *C. sativus*. In pachytene and prometaphase I eight bivalents were present. In metaphase of Divisions II, III, and IV, six, seven, and eight chromosomes were counted. It may therefore be concluded that the haploid complement is at least six, and is probably eight. In the conjugate division of the croziers at least seven deeply staining bodies,

i.e. prochromosomes, were observed; the same number appeared in the nuclei of the stromatic tissue. If each prochromosome represents an individual chromosome, then these observations indicate the existence of a haploid complement of seven or eight. Although Shoemaker (7) stated that the haploid chromsome complement in this fungus is six, he included no photographic evidence. The present study showed that there are at least three small chromosomes. If Shoemaker's counts were made on metaphase figures, it is possible that some of these smaller chromosomes, which measure less than 0.5 μ at this stage, may have been missed. Indeed, in certain asci in metaphase I the author could also resolve only six chromosomes. Chromosome counts, based on two figures each, of metaphase I of a 47 $\times$ T_2 (*A*) mating and metaphase II of a 238 $\times$ A_{18} (*a*) mating were six and eight, respectively. Such a discrepancy may be due to overlapping of chromosomes as suggested above or to some form of structural hybridity which entailed an apparent decrease in the number of separate chromosome entities.

It is impossible to make any generalizations regarding chromosome morphology, since the study was based exclusively on the mating T_2 (*A*) $\times$ A_{18} (*a*). From the pachytene figures in asci of this mating, it appears that there are eight chromosomes, two of which are quite long and three of which are quite small. Between these two extremes there are three chromosomes which are about one half the length of the long chromosomes. Of the two long chromosomes which may measure 16 μ or more at mid pachytene, one (chromosome 1) may appear in the form of an asymmetric 'T' configuration or as a cross. It is possible that the 'T' configuration is basically a cross that has been poorly flattened. Chromosome 4 usually appears as a symmetrical cross. The author is convinced that the presence of these crosses is not due to overlapping of two separate bivalents. The occurrence of these crosses in pachytene, as well as the presence of chromosomes free of the spindle in metaphase III, and the trailing of chromosomes in anaphase III and IV, together with the observation that the nuclei in telophase III may vary considerably in the amount of stainable chromatin material, is evidence of structural hybridity, probably involving reciprocal translocations. The cross configurations in the pachytene nuclei would on this interpretation represent two bivalents. Abnormal disjunction of such configurations (tetravalents) could well account for the meiotic and mitotic abnormalities listed above; the occurrence of abnormal and degenerating ascospores would be a manifestation of 'segregational sterility', since ascospores are equivalent genetically to gametes of higher organisms. Cross configurations may also represent normal bivalents with single chiasmata. If such were the case then rotation of the centromeres and terminalization of chiasmata in *C. sativus* must occur in pachytene and not in the later meiotic stages as it occurs in *Neurospora* (9) and the higher plants and animals. In his cytological study of *C. sativus*, Shoemaker (7) mentioned that the late pachytene bivalents gave no evidence of structural hybridity.

The study of chromosomal behavior in *C. sativus* shows that it does not differ radically from that reported for *Neurospora crassa*. McClintock (4) and later Singleton (9) found that the chromosomes in the fusion nucleus of the young ascus of *N. crassa* do not synapse at a state of maximum extension as in higher organisms, but rather at a state of maximum contraction. The synapsed chromosomes, i.e., the bivalents, then begin to elongate with the enlarging ascus. The author found the same behavior in *C. sativus*. In his study of *Pyronema confluens*, McIntosh (6) had no clear indication that synapsis was initiated between highly contracted chromosomes. From an examination of presynaptic stages of *C. sativus* it appears that some of the chromosomes at least are present as double strands, in which case an early pachytene bivalent may actually be four stranded. Such observations were not obtained by McClintock (4) or Singleton (9) for *N. crassa*. Following late pachytene the bivalents begin to contract. However, the author could not find any figures comparable to Fig. 34 of Singleton (9), which he called mid diakinesis and in which chiasmata were evident. Like *N. crassa*, *C. sativus* contains at metaphase I a truncated spindle with prominent astral fibers radiating from a rodlike centriole. In late telophases and in interphases of the various divisions heterochromatic regions were present. However, these were irregular in shape and not beaked as in *Neurospora* spp. (9). In Divisions II and III the spindle was elliptical and possessed dotlike centrioles. Singleton (9) found that for *N. crassa* the centriole increased in size between metaphase I and the later divisions and appeared as a triangular plate. Astral fibers were quite prominent in all division figures in *C. sativus*, and resembled those found in *N. crassa*.

Tinline (11) found that light was detrimental to ascospore delimitation. The present study suggested that structural hybridity of the chromosomes may also prevent ascospore delimitation and maturation. In this connection cytological studies of Shoemaker's completely sterile matings 104*c* × 104*d* might be very revealing, since he (7, 8) found that with these isolates spores were never delimited although numerous young asci were present. Since *C. sativus* possesses large asci and lends itself well to the squash staining techniques, it is hoped that future endeavors may be directed to cytology of the ascus of such matings.

Acknowledgments

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References

1. HRUSHOVETZ, S. B. Cytological studies of *Helminthosporium sativum* and its perfect stage *Cochliobolus sativus*. Ph.D. Thesis, University of Toronto, Toronto, Ont. 1955.
2. HRUSHOVETZ, S. B. Cytological studies of *Helminthosporium sativum*. Can. J. Botany, 34 : 321-327. 1956.
3. KURIBAYASHI, K. The ascigerous stage of *Helminthosporium sativum*. Trans. Sapporo Nat. History Soc. 10 : 138-145. 1929.
4. MCCLINTOCK, B. Neurospora. I. Preliminary observations of *Neurospora crassa*. Am. J. Botany, 32 : 671-678. 1945.
5. MCINTOSH, D. L. A Feulgen-carmine technic for staining fungus chromosomes. Stain Technol. 29-31. 1954.
6. MCINTOSH, D. L. A cytological study of ascus development in *Pyronema confluens* Tul. Can. J. Botany, 32 : 440-446. 1954.
7. SHOEMAKER, R. A. Biology, cytology and taxonomy of *Cochliobolus sativus* states of *Helminthosporium* species. Ph.D. Thesis, Cornell University, Ithaca, N.Y. 1955.
8. SHOEMAKER, R. A. Biology, cytology, and taxonomy of *Cochliobolus sativus*. Can. J. Botany, 33 : 563-576. 1955.
9. SINGLETON, J. F. Chromosome morphology and the chromosome cycle in the ascus of *Neurospora crassa*. Am. J. Botany, 40 : 124-144. 1953.
10. TINLINE, R. D. Studies on the perfect stage of *Helminthosporium sativum*. Can. J. Botany, 29 : 467-478. 1951.
11. TINLINE, R. D. Physiology, pathogenicity, and genetics of *Helminthosporium sativum*. Ph.D. Thesis, University of Wisconsin, Madison, Wis. 1954.

STUDIES ON THE SEEDLING DISEASE OF BARLEY CAUSED BY HELMINTHOSPORIUM SATIVUM P.K. & B.[1]

By R. A. Ludwig,[2] R. V. Clark,[3] J. B. Julien,[3] and D. B. Robinson[4]

Abstract

A standard sand – cornmeal – nutrient salt medium, for use in the production of artificial inoculum of *Helminthosporium sativum*, is described. This inoculum induces uniform plant disease development when thoroughly incorporated with the planting soil. The results presented clearly demonstrate the necessity of using a series of infestation levels in studies of factors affecting disease development in artificially infested soil. It is shown that considerable reliance can be placed on treatment comparisons within an experiment but that comparisons between experiments are much less accurate. The role of a toxin (or toxins) in disease development in barley seedlings has been demonstrated. The toxic activity was found to be distinct from that frequently encountered on addition of organic matter to soil. Results obtained suggest that toxin adsorption by the soil may play an important role in reducing disease incidence and severity.

Helminthosporium sativum produces a seedling blight, foot and root rot, head blight, and leaf spot of cereals and grasses (8). These various phases of the disease are responsible for a steady annual loss in crop yield (3, 18). The pathogen is either seed or soil borne but is not a vigorous soil saprophyte and, under arid conditions at least, soil invasion would seem to be limited to dormant conidia and infested plant debris (26). Information on the physiology of the disease is largely confined to scattered observations reported in connection with ecological studies.

The work reported in this paper is part of a study of the physiology of disease development particularly as related to seedling invasion. It consists firstly of a re-evaluation of methods of disease induction and assessment, and secondly, of a study of the relationship of toxic substances to disease development.

Inoculation Methods

The various methods used to obtain root inoculation with *H. sativum* were reviewed by Sallans (22). These methods consisted of the artificial infestation of seeds with the fungus prior to planting and the artificial infestation of the planting soil either by the introduction of spores or whole cultures of the fungus. In the latter case inoculum consists of the fungal growth plus its substratum. A variety of media have been used for growing the fungus in the preparation of whole culture inocula. These media consist of wheat, oat, and barley mixtures, oats, oat hulls, oatmeal, rice, and sterile soil.

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The practicability of artificial barley seed inoculation as a means of inducing disease was tested. A heavy spore suspension of *H. sativum* was prepared from potato dextrose agar cultures and seed of the varieties "Star" and O.A.C. 21 soaked in it for half an hour. Following soaking, the seed was drained and incubated in a moist chamber at room temperature. Samples were withdrawn at intervals of 0, 3, 7, 11, and 22 hr. and immediately air-dried. Checks were treated similarly except that the seed was soaked in water instead of a spore suspension. Planting was done in 3 × 3 in. plant bands* containing a 3 to 1 greenhouse compost : sand mixture at a rate of 25 seeds per band. Notes were taken 10 days after planting.

Disease development was negligible after the 3 and 7 hr. incubation treatment and only moderate infection occurred following the 11 and 22 hr. incubation period. In the latter case the variation in disease development between plants within treatments was large. This means that certain seeds were more effectively infested than others. Disease development was largely confined to a lesioning of the coleoptile, most of the roots being free from infection.

The application of spores to the soil at the time of planting was next tried as a means of producing disease. Spore suspensions ranging in concentration up to 1500 spores per ml. were prepared and applied to the soil at the rate of 5 ml. per 6 cu. in. of soil immediately after planting. At the end of 10 days all the plants were completely free of root rot. A few scattered lesions appeared on the coleoptiles of some plants growing in the most heavily infested bands. No growth differences could be observed.

In another experiment a dry spore mass was obtained using the method described by Sherf *et al.* (24) for *Helminthosporium oryzeae*. Dry barley seeds were pelleted with this using methyl cellosolve as a sticker (14). Seeds, in the center of a ball of viable spores approximately $\frac{1}{4}$ inch in diameter, were planted in vermiculite. The resulting seedlings were completely disease free and more vigorous than the controls from non-pelleted seed.

The feasibility of artificial soil infestation with mycelial suspensions of *H. sativum* was tested. The fungus was grown for two weeks on a liquid potato dextrose medium. The mycelial mat was then removed by filtration and broken up in a Waring blendor in a minimum of tap water. The resulting suspension was mixed uniformly with soil in a series of concentrations ranging from 0 to 50% by volume of the soil used. Seed of the varieties O.A.C. 21 and Star was planted at each level of infestation. No growth differences could be observed among the treatments after 10 days. The root infection of both varieties was about 8% at the highest level of infestation; again coleoptile lesioning was more prominent than root invasion.

A comparison was next made of the disease producing capacity of inocula produced by growing the fungus on a number of organic media used by other workers. The media chosen for this comparison were greenhouse compost,

**Obtained from Bird & Son, Inc., East Walpole, Mass., through W. H. Peron & Co., 955 St. Lawrence Blvd., Montreal.*

greenhouse compost + 10% cornmeal, cornmeal, wheat (unground), and oat hulls obtained by coarsely grinding oats and screening out approximately one-third of the meal. These five materials were dispensed in shallow layers in flasks moistened with water and sterilized by autoclaving. On cooling, each flask was inoculated with 2 ml. of a spore suspension of the fungus and incubated for two weeks at 26° C. The resulting inocula were mixed uniformly with greenhouse compost at rates of 5% and 15% by volume for planting in 3 × 3 in. plant bands. For purposes of comparison similar bands filled with greenhouse compost were included. Notes were taken after 10 days of growth. The results are presented in Table I.

TABLE I

THE EFFECT OF TYPE OF INOCULUM ON DISEASE PRODUCTION IN BARLEY SEEDLINGS

Type of inoculum	Percentage soil infestation	Percentage emergence	Percentage disease*	Average height (cm.)
Soil	5	99	4	15.3
	15	99	8	15.0
Soil–cornmeal	5	98	21	13.2
	15	93	74	8.4
Cornmeal	5	83	77	7.9
	15	26	100	4.1
Wheat	5	95	33	16.0
	15	92	62	13.3
Oat hulls	5	91	36	15.3
	15	91	74	10.8
None	—	83	0	16.0

**Based on McKinney's numerical rating system (19).*

Soil alone proved to be a poor medium for the growing of inocula. This was apparently due to the poor growth of the fungus supported on the greenhouse compost used. Cornmeal was unsuitable because it formed a sticky paste in the culture flasks and was difficult to break up when it was being mixed with soil. It will be noted that the addition of cornmeal to the planting soil caused a serious reduction in emergence, an effect difficult to attribute entirely to the organism itself. The wheat and oat hull inocula seem, from the data presented, to be less potent than the soil–cornmeal inoculum. This effect appeared to be due to the greater variability between plants within treatments when these inocula were used. Some plants escaped the disease completely while others were severely damaged. Plants within treatments behaved much more uniformly when the soil–cornmeal inoculum was used. These differences may be explained on the basis of particle size differences. With the large particles, such as occured in the whole-wheat inoculum, certain seeds contacted the inoculum while others did not. With the small particle size occurring in the soil–cornmeal inoculum all seeds and seedlings were equally exposed to infection.

In an attempt to further standardize the inoculum a comparison was made between the soil–cornmeal inoculum and an inoculum grown in a sand – nutrient salt – cornmeal medium. In the latter case coarse sand containing 5% cornmeal was dispensed into flasks, moistened with a nutrient solution containing the inorganic salts used in Czapek's medium, and then sterilized by autoclaving. A comparison of the disease development at two levels of soil infestation is given in Table II.

TABLE II

A COMPARISON OF THE EFFECTIVENESS OF SOIL–CORNMEAL INOCULUM AND SAND – CORNMEAL – NUTRIENT SALTS INOCULUM IN INDUCING DISEASE IN BARLEY

Type of inoculum	Percentage soil infestation	Percentage disease	Average height (cm.)
Soil–cornmeal	4	13	12.6
	12	77	7.4
Sand – cornmeal – nutrient salt	4	22	11.8
	12	64	8.9
None	0	0	12.9

No great differences were observed in the activities of the two inocula. The sand inoculum, however, was easier to handle and mixed more uniformly with the planting medium than did the soil inoculum and for this reason resulted in greater uniformity of disease development. The latter effect was especially noticeable where low levels of soil infestation were used. It was concluded that the adoption of a sand – cornmeal – nutrient salt medium for the production of inocula would add to the ease and accuracy of comparisons as well as reduce the variability in composition of the culture medium from one batch to another. Inoculum used in the succeeding experiments reported in this paper was grown on this medium in 500 ml. widemouth Erlenmeyer flasks unless otherwise specified. Before being incorporated with the planting soil, it was removed from the flasks and rubbed through a sieve made of fly screening. The incorporation of the inoculum into the planting soil could be accomplished more uniformly by gradually adding the soil to the inoculum during the mixing. This was particularly important when small amounts of inoculum were being used to produce low levels of soil infestation.

The Effect of Incubation Time on Activity of Sand–Cornmeal Inoculum

A series of experiments were undertaken to determine the age of inoculum resulting in maximum disease development. Results of preliminary experiments indicated that an incubation period of 7 to 14 days gave maximum activity. In the final experiment of the series, a number of flasks of sand – cornmeal – nutrient salt medium were inoculated at intervals over a 30 day period so that inocula of varying ages were available for infesting soil at a single planting time. All flasks were incubated at approximately 24° C. As

in previous experiments O.A.C. 21 barley was planted in soil uniformly mixed with the inoculum, an inoculum level of 15% by weight being used. The ages of inoculum used and results obtained in one replicate are illustrated in Fig. 10.

These results confirm those of preliminary experiments and show that maximum disease producing activity occurs in the 7- to 10-day-old inoculum.

Location of Inoculum in Relation to Disease Development

The amount of an inoculum mixture introduced in soil and the location of the mixture with reference to the seed may effect the intensity of the disease that subsequently develops. An experiment was conducted to find the effect of location of inoculum on disease development. One part of soil–cornmeal inoculum was mixed with nine parts of greenhouse compost and layers of this infested soil introduced in plant bands in various ways. Planting was done at a depth of 1 inch below the soil surface and all treatments were replicated four times. The method of adding infested soil and the results obtained after 10 days' growth are given in Table III.

TABLE III

LOCATION OF INOCULUM IN RELATION TO DISEASE DEVELOPMENT

Method of adding infested soil	Average height (cm.)	Percentage disease
Check—no infested soil added	13.3	0
Infested soil in bottom inch of band—non-infested soil above and below seed	12.8	20
Infested soil in top and bottom inches of band—non-infested soil immediately below the seed	10.7	35
Infested soil in center inch below the seed—non-infested soil above the seed and the bottom of the band	8.2	79
Infested soil in bottom two inches of band—non-infested soil above the seed	7.7	80
Infested soil above and below seed—non-infested soil in the bottom inch of the band	6.0	94
Infested soil completely filling band	5.7	84

These results show that a layer of infested soil immediately below the seed is necessary for heavy root infection. Where infested soil occurred above the seed only, or was separated from the seed by a layer of non-infested soil, comparatively little disease developed.

Population Size, Degree of Replication, and Methods of Note Taking

In the early work reported in this paper planting was done at a rate of 25 seeds per 3 × 3 in. plant band. Individual treatments were replicated between 5 and 10 times. Disease incidence was measured by either a numerical rating as suggested by McKinney (19) or by measuring the plant height. A statistical

comparison of these two types of data gave a correlation coefficient of −0.979, a figure which was highly significant. In the latter case, the height of individual plants from the seed to the tip of the longest leaf was measured, and the resulting figures averaged to obtain the average plant height per band. This procedure proved too cumbersome for use in a large experiment. An experiment on the size of plant population and degree of replication necessary to give sufficient accuracy to the test was therefore undertaken. Various methods of note taking were tested at the same time in order to establish as simple a procedure as possible.

In this experiment ordinary greenhouse soil was used as the planting medium. Planting was done at two rates, namely, 10 and 25 seeds per band. A series of concentrations of inoculum ranging at 5% intervals from 0 to 30% by weight were used. All treatments were replicated 10 times and placed at random within each replicate. Notes were taken after 10 days. Three methods of determining the plant height per band were employed. These consisted of (*a*) placing a ruler against the band and estimating the mean height per band, (*b*) placing a ruler against the band estimating the height of each seedling and averaging to get a mean for the band, and (*c*) washing the roots free of soil, measuring the height of each plant from the seed to the tip of the longest leaf and averaging to get a mean for the band. The analysis of variance of the data on height obtained after the seedlings were washed free from soil and measured is presented in Table IV.

TABLE IV

ANALYSIS OF VARIANCE OF HEIGHT DATA FOR SEEDLINGS WASHED FREE OF SOIL

Source of variance	D.F.	S.S.	M.S.	F.	5%
Between seed numbers					
Seed numbers	1	0.25	0.25	0.27	5.12
Replicates	9	25.13	2.79	3.00	
Error (*a*)	9	8.39	0.93		
Within seed numbers					
Rates of soil infestation	6	2033.84	338.97	371.49	2.27
Rates × seed numbers	6	3.83	0.64	0.70	2.27
Error (*b*)	108	98.27	0.91		
Total	139	2169.71			

From these results it is obvious that a reduction in the number of seeds from 25 to 10 per band makes little difference to the accuracy of the test. This simplification reduces the amount of work involved as well as the quantity of seed required; it also permits the use of smaller plant bands and consequently a substantial reduction of the amount of inoculum required for the test. The difference required for significance between rates of soil infestation at the 5% point was calculated for varying number of replicates. It was found that the error could be reduced to approximately 10% by the use of five replicates. Further replication, as would be expected, does not result in

a further proportional decrease in error. It was decided, therefore, to adopt this as a reasonable error and to use this number of replicates as the standard procedure.

The estimates of mean plant height per band were compared with the figures obtained after washing and measuring by calculating correlation coefficients based on five replicates.

A correlation coefficient of 0.98 for the general estimate and 0.998 for the individual plant estimate was obtained. In view of this high correlation it is obvious that the simple method of estimating the general means for a band can be adopted as a standard procedure. The results of this experiment show that a high degree of uniformity can be obtained within a single experiment but are apt to be misleading in so far as comparisons between experiments are concerned. This is illustrated in Fig. 1 where the means of eight experiments conducted in the greenhouse over a period of several months have been averaged and are plotted along with lines marking the 5% probability limits. It will be noted particularly that variability is approximately equal throughout the range of soil infestation used. This suggests the effect of some factor which is operating independently of the disease.

Comparisons of soil infestation levels in a number of experiments indicated that intervals of 2% yielded significantly different results but that the technique did not permit differentiation between closer intervals.

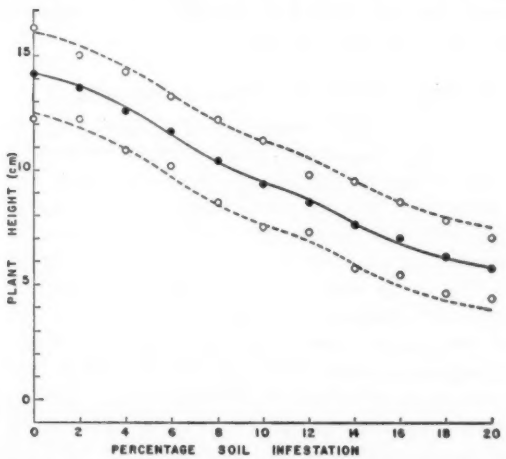


FIG. 1. Disease development in relation to soil infestation with 5% probability limits.

Effect of Seedling Vigor as Influenced by Seed Size on Disease Development

A comparison was made of the effect of seedling vigor on disease incidence within a single experiment. Four seed size grades were obtained by passing a uniform sample of O.A.C. 21 barley through a series of slotted screens. Seeds of each grade were then used for planting over a soil infestation range. The

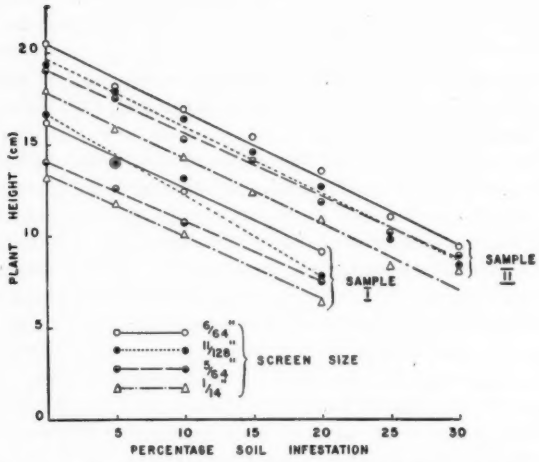


FIG. 2. Effect of seed size on seedling vigor and disease development.

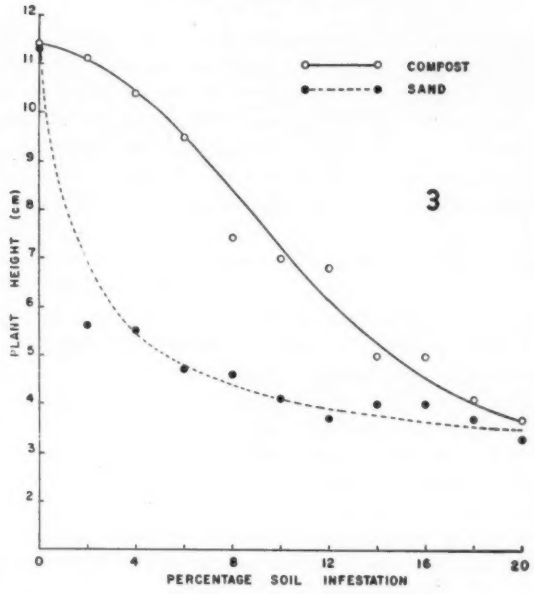
results obtained with two such samples planted at different times are presented in Fig. 2. It will be noted that decreasing seed size resulted in decrease in vigor but that all lines are essentially parallel. This indicates a uniformity of response that is independent of vigor.

The Effect of Planting Medium on Disease Development

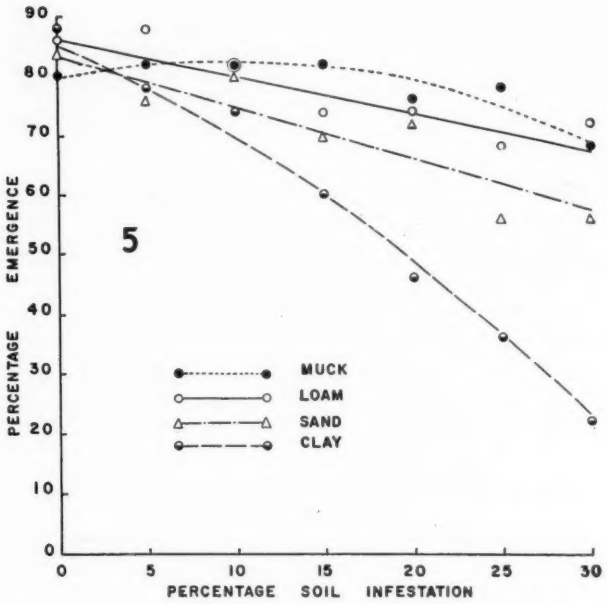
Quartz sand and greenhouse compost were compared as planting media in an initial experiment. The results, Fig. 3, show that although the plant height in the controls was approximately equal, the behavior in the two media was radically different. The plants in sand were characterized by a large initial drop in plant height followed by a gradual decline to the maximum level of soil infestation used, while the plants grown in compost declined gradually over the entire range of soil infestation used, the curve being somewhat sigmoid in character.

A wood sand, black muck, heavy clay, and Greensboro loam were compared in a second experiment. These were selected because of their variability in organic matter content and physical characteristics. The effect of level of soil infestation on plant height is given in Fig. 4. Disease development was least in the muck and greatest in the clay, with loam and wood sand being intermediate and approximately equal. The height data in Fig. 4, represent a mean of the emerged seedlings. Difference in emergence, Fig. 5, occurred in the same order.

The fact that differences between soils were not due to different levels of microbiological antagonism was demonstrated in two experiments. In the first of these, disease development was compared in sterilized and unsterilized soil, all soils being infested with inoculum at a level of 10% by weight. From



FIGS. 3 and 4. Relative disease development in sand and greenhouse compost (Fig. 3) and in four soil types (Fig. 4).



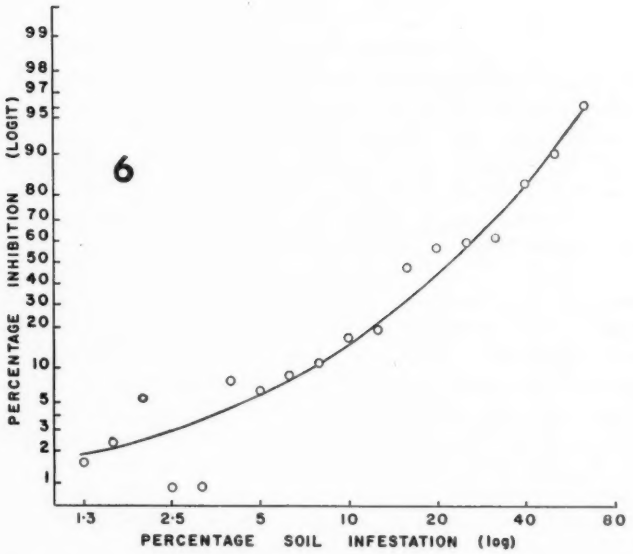


FIG. 5. Relative seedling emergence in four types of artificially infested soil.
FIG. 6. The *Helminthosporium* disease curve.

the results illustrated in Fig. 12 it is apparent that soil sterilization made little difference to disease incidence and that it did not affect the order of the soils in this regard. In the second experiment a series of flasks of seven-day-old inoculum were contaminated with 5 ml. of air-dry soil of each of the types employed in the preceding experiments. These were incubated for a further five days and used to infest soil at a level of 20% by weight. It is obvious from Fig. 11 that the activity of the inoculum was unaffected.

The Relationship of Toxic Substances Produced by H. sativum to Disease Production in Barley Seedlings

The curves presented in the preceding section suggest a simple sigmoid relationship (see Fig. 2) between amount of inoculum added to soil and resultant disease incidence. In order to confirm this relationship an experiment was undertaken in which soil infestation levels ranging at logarithmic intervals from 0 to 77% were employed. Planting was done in a sandy loam soil with particular care being taken to obtain uniformity of infestation and depth of planting. Disease data were recorded as height of emerged plants after a growth period of 10 days. Emergence, in this experiment, was approximately equal over the soil infestation range used. Averages were plotted and the theoretical maximum and minimum plant heights were obtained by extrapolation. Using these figures the data were transformed to percentage growth inhibition by means of Abbott's formula (10). The results, plotted on a logit–log grid are presented in Fig. 6.

It will be noted that a smooth curve was obtained instead of the anticipated straight line. This is not surprising in view of the arbitrary manner employed in obtaining maximum growth inhibition. The result does, however, indicate a simple relationship between soil infestation and disease incidence and could easily be explained by the action of a toxic substance on the plant. Considerable variability is apparent at low levels of soil infestation and raises some questions as to the validity of the curve in this region. The points however represent very small differences in level of soil infestation and, as previously indicated, differences between infestation levels of less than 2% are difficult to establish. In many experiments a plant growth stimulation occurred in this region. This might explain the stimulated yields sometimes obtained (23) as a result of artificial inoculation. Such an effect provides further evidence for a direct chemical effect.

More direct evidence for the activity of toxins in the development of disease was obtained by experiments in which inoculum was leached with water to remove such materials without reducing the amount of fungus present. Several experiments in which inoculum was washed prior to infestation of the soil gave inconclusive results. It was felt that the indifferent results might be caused by the production of more toxin, subsequent to the infesting of soil, in sufficient quantities to give almost the same amount of disease development as was secured by using unwashed inoculum. With this in mind an experiment was conducted in which the growth period of the seedlings in the

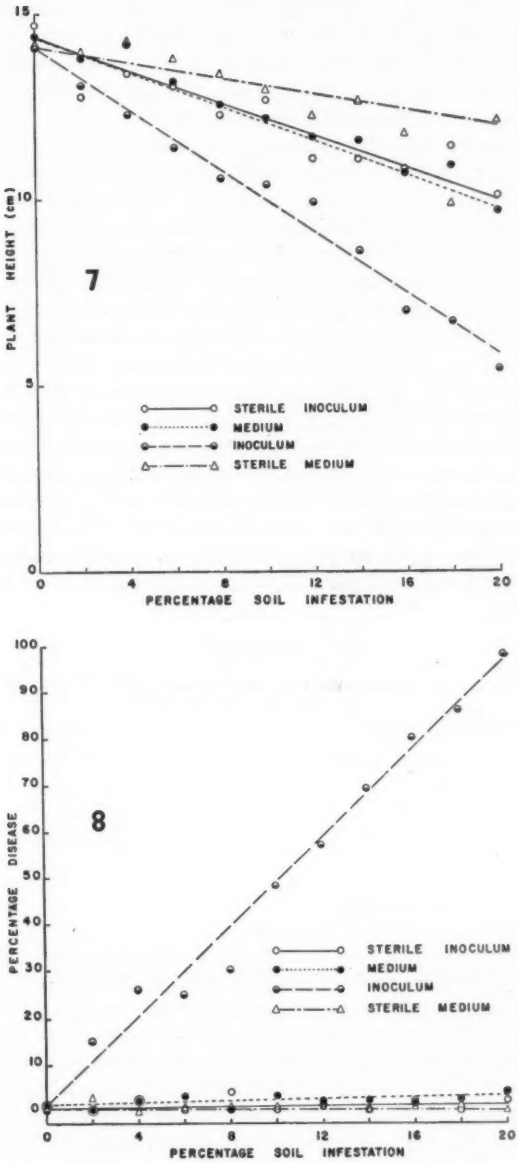
infested soil was reduced by pregermination of the seed. Unwashed and water-washed inoculum were used to infest greenhouse compost at a rate of 10% by weight of compost used. These soils, together with a non-infested check, were planted with seedlings previously germinated on blotting paper for three days. The results obtained after a further seven days' growth are illustrated in Fig. 13.

The disease obtained in the control, washed inoculum, and unwashed inoculum was 0, 60, and 100% respectively. In a second similar experiment the effect of leaching was studied in plantings made in sand in Büchner funnels. Duplicate series, one planted with dry seed and one planted with seed sprouted as in the previous experiment, were used. The washing treatment consisted of an initial leaching, prior to sand infestation, with an excess of water followed by supplementary leachings with approximately 100 ml. of water twice daily during the growing period. Excess water was removed by suction following each treatment. A uniform moisture content was maintained throughout the series by also applying suction to the "non-washed" treatments twice daily and saturating them with water without permitting "run-off". The results of this experiment are shown in Fig. 14. Frequent checking of leachings showed that the fungus was not washed out of the planting medium during the course of the experiment. Both experiments demonstrate the essentiality of a water-soluble substance (or substances) for disease development.

Further evidence for toxins in active inoculum was obtained through the effect of leachates on seed germination. In one of these experiments 100 gm. of 10-day-old inoculum was extracted with 100 ml. of water. Barley seed was then soaked in the extract for 24 hr. at room temperature, removed, and placed to germinate in soapstone germination dishes. A similar sample of seed was soaked in water as a control. After three days only 6% of the seeds treated with extract had germinated while 84% had germinated in the control. Similarly treatment with filtrate from a 10-day-old culture of *H. sativum* on a potato dextrose liquid medium resulted in a germination of only 2% while the control germinated 85%.

Toxin Production vs. Toxic Media

The toxicity of the medium employed in preparation of inoculum is frequently pointed out in connection with studies of this kind (22, 29). A series of experiments were undertaken in order to clarify this point in connection with these studies. In an initial experiment sterilized and unsterilized sand – cornmeal – nutrient salt medium, as well as sterilized and unsterilized inoculum prepared by growing the fungus for 14 days on this medium, were used to amend soil at a series of levels. Sterilization was accomplished in this case by autoclaving for five hours at 15 lb. pressure. Planting was done in the usual manner. The results expressed in terms of plant height and percentage disease are given in Figs. 7 and 8 respectively. While it is apparent that some depression of plant height occurred as a result of adding the medium to the soil, this effect was small compared to that produced by



FIGS. 7 and 8. A comparison of the effect of sterile (autoclaved for five hours) and non-sterile medium and inoculum on barley seedling growth (Fig. 7) and on disease development in barley seedlings (Fig. 8).

live inoculum. Differences are more striking when evaluated by a numerical disease rating. The behavior of sterile inoculum was similar to that of the original medium suggesting that toxic substances were either destroyed in the sterilization process or were inactive in the absence of the viable fungus. In a second similar experiment 10-day-old inoculum was sterilized by autoclaving at 15 lb. pressure for one hour, in contrast to the previous five-hour period, and used to infest soil at 0, 4, 10, and 20%. The results obtained are illustrated in Fig. 15.

Depression of plant height and increase of root injury accompanied increase of the level of soil infestations and was very extensive at the 20% level. Plantings from these roots yielded *Phythium* spp., *Fusarium* sp., and a variety of unidentified *Phycomycetes*, but no *H. sativum*. Repetitions of this experiment confirmed these findings. It must be concluded, therefore, that *H. sativum* produces a water–soluble toxic substance (or substances) with at least a degree of heat stability and that the activity of this substance is distinct from toxicities often manifest when organic media are added to soil.

Samples of filtrate from two-week-old cultures of *H. sativum* on liquid potato dextrose medium were autoclaved at 15 lb. for varying periods of time. Their toxicity as measured by an effect on seed germination was determined. Seeds were soaked for 24 hr. and then placed in soapstone dishes for germination. The results are given in Table V. In this case it is apparent that the active principle was partially destroyed by a one hour heat treatment in the autoclave.

TABLE V

EFFECT OF AUTOCLAVING ON SEED GERMINATION INHIBITION BY CULTURE FILTRATES

Autoclaving time (hr.)	% Inhibition (3 days)
0	97
1	35
5	31
Water control	13

Effect of Soil Contact on Toxicity of Culture Filtrates of H. sativum

A series of experiments were undertaken to determine the effect of soil contact on the toxicity of culture filtrates of *H. sativum*. The filtrates used were from 10-day-old cultures of the organism grown in a potato dextrose liquid medium in still culture. Activities were evaluated by means of the barley seed germination test. Seeds were soaked in the test solutions for eight hours and then transferred to cheesecloth wicks placed over screen supports in Petri dish germinators containing water. Results are expressed as percentage non-germination after three days. Soils of the four types previously referred to (Figs. 5 and 6) were used in these experiments.

Equal volumes of air-dry soil were placed in columns, in the first experiment, and filtrate passed through them. The weight of each soil involved, and the amount of filtrate required to just saturate it, is given in Table VI.

Thirty milliliter samples were collected for assay. The first sample was the initial run off. One hundred milliliter quantities were discarded between each of the succeeding samples. The results of this experiment are given in Table VII.

These results suggest that the toxic substances produced by *H. sativum* were adsorbed by the soil and that the adsorptive capacity of the loam and wood sand was much less than that of the muck and clay. The rate of percolation was, however, much slower in the case of the latter two soils and ceased altogether before a third sample could be collected, a period of two days being required to obtain the second sample from the clay column. This would allow ample time for decomposition as a result of microbial activity.

A second experiment was conducted in which equal volumes of soil (see Table VI) were suspended in 100 ml. quantities of filtrate in quart fruit jars. The soils were kept in suspension by placing the jars on a rotary shaker with an "end over end" motion. Jars of each soil type were removed from the shaker at intervals. The liquid was separated from the soil by filtration and its effect on barley seed germination tested. The length of soil contact and activity results are given in Table VIII.

TABLE VI

AMOUNT OF AIR-DRY SOIL ADDED TO COLUMNS AND VOLUME OF FILTRATE REQUIRED FOR SATURATION

Soil type	Soil in column (gm.)	Filtrate required for saturation
Loam	65.0	60.0
Muck	26.0	40.0
Wood sand	63.5	60.0
Clay	60.5	50.0

TABLE VII

EFFECT OF PASSAGE THROUGH SOIL ON TOXICITY OF CULTURE FILTRATES

Sample	Percentage germination inhibition			
	Loam	Muck	Wood sand	Clay
1st 30 ml.	−7*	0	0	8
130–160 ml.	34	14	55	15
260–290 ml.	59	—	80	—
Filtrate	79	76	83	85

**Figures corrected for non-germination in water control.*

TABLE VIII

EFFECT OF TIME ON INACTIVATION OF CULTURE FILTRATES BY SOILS OF DIFFERENT TYPES

Time of exposure to soil (min.)	Percentage germination inhibition			
	Loam	Muck	Wood sand	Clay
0	64	68	60	62
7.5	18	30	24	48
15	16	34	22	36
30	18	34	12	38
60	18	28	14	36
120	12	22	18	30
240	14	24	18	20

The inactivation of the filtrates occurs rapidly following contact with soil and is relatively independent of time. This is as would be expected if adsorption phenomena were involved.

In a final experiment soil samples of the four types were placed in contact with varying amounts of culture filtrate for a period of one hour and then removed by filtration and tested. The volume of soil was the same as that used in the previous experiments. The results are presented graphically in Fig. 9.

These results, together with the observed relative independence of time, provide good evidence for the adsorption of the toxic principle by the soil. It will also be noted (see Fig. 5) that the maximum adsorption occurred in the muck soil where disease development was least.

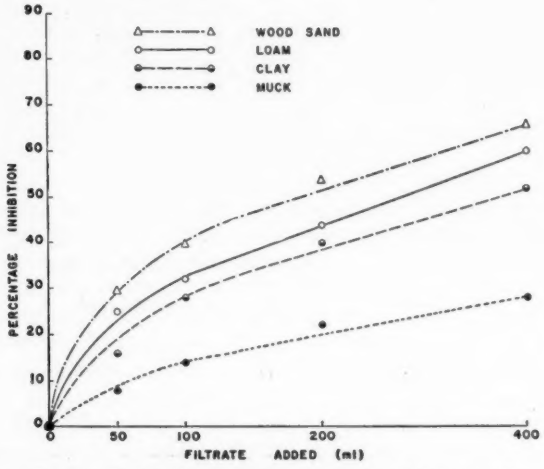


FIG. 9. Toxin adsorption by soil samples of four types.

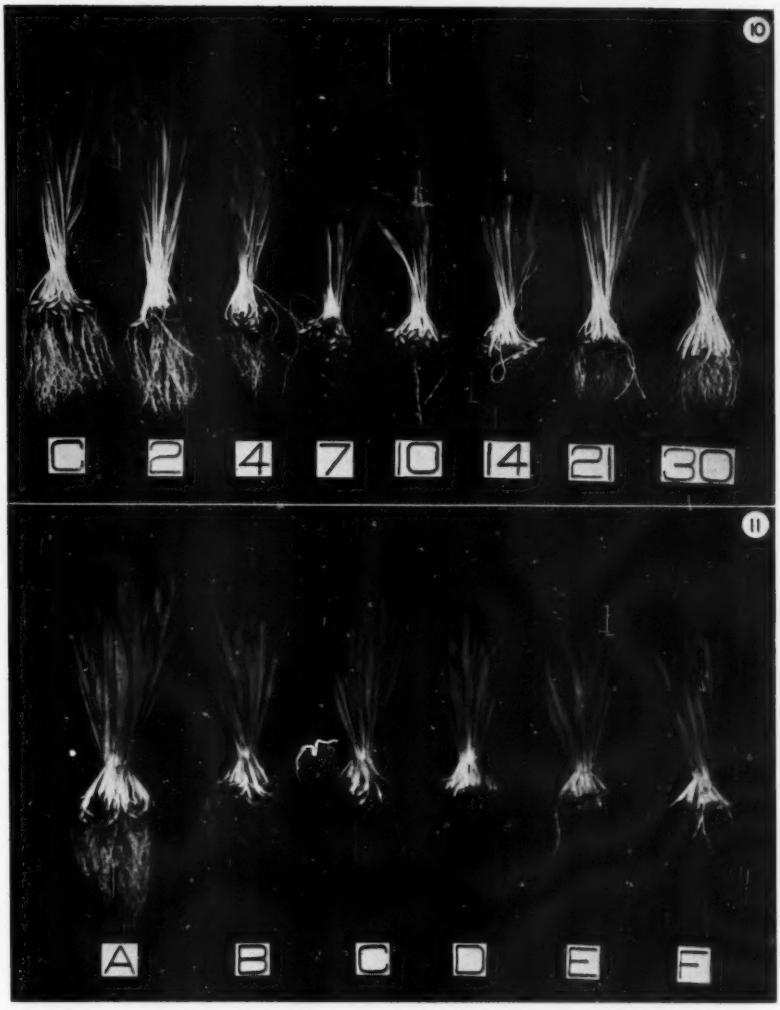


FIG. 10. Disease development in barley seedlings grown in soil artificially infested with inoculum of different ages. Numbers refer to the age, in days, of the inoculum added to the soil in which the plants were grown.

FIG. 11. Disease development in barley seedlings grown in soil infested with inoculum previously contaminated with different soils. A—check, no inoculum; B, C, D, and E—inoculum contaminated with muck, clay, sand, and loam respectively; F, uncontaminated inoculum.

FIG. 12. Disease development in barley seedlings grown in three soil types sterilized before planting. A—loam; B—clay; C—muck. 1. Unsterilized, non-infested soil. 2. Sterilized, non-infested soil. 3. Unsterilized, infested soil. 4. Sterilized, infested soil.

FIG. 13. Disease development in barley seedlings grown in soil infested with washed and unwashed inoculum. A—check, no inoculum; B—washed inoculum; C—unwashed inoculum.

1. Unsterilized, non-infested soil. 2. Sterilized, non-infested soil. 5. Unsterilized, infested soil.

FIG. 13. Disease development in barley seedlings grown in soil infested with washed and unwashed inoculum.
A—check, no inoculum; B—washed inoculum; C—unwashed inoculum.

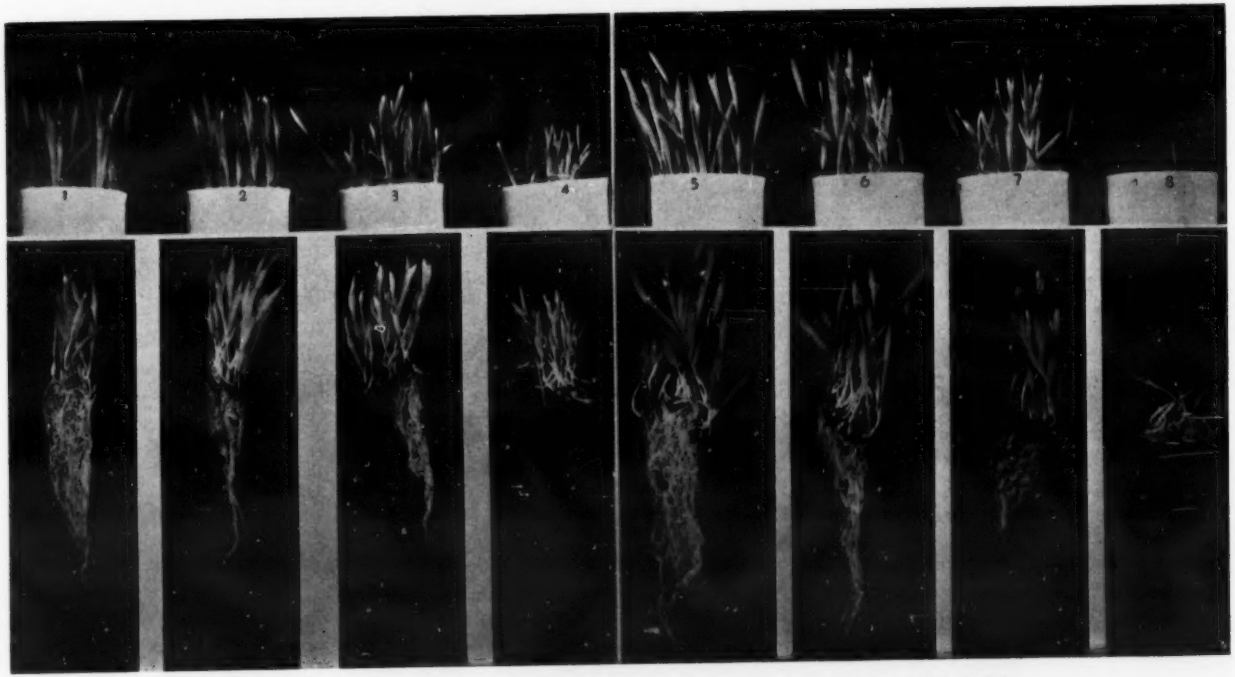


FIG. 14. Effect of leaching on disease development in barley seedlings grown in infested soil.
1 to 4—sprouted seed; 5 to 8—dry seed. 1 and 2, 5, and 6—check, leached, and not leached respectively. 3 and 4, 7, and 8—inoculated, leached, and not leached respectively.

FIG. 15. Disease development in barley seedlings grown in soil artificially infested with inoculum sterilized by autoclaving for one hour. Numbers refer to the percentages of inoculum added to the soil.

A series of experiments were conducted in which culture filtrates were treated with aqueous leachates from soil. In no case were these found to reduce the toxicity of the filtrates. Similarly treatment of filtrates with inert clays such as S.P.V. Volclay and Barden clay were without effect. This provides further indication that inactivation occurs as a result of adsorption on the organic matter of the soil.

Discussion

Attempts to induce a satisfactory disease incidence in roots through artificial seed infestation with *H. sativum* were unsuccessful. Artificial infestation of soil with either spores or mycelium also proved to be unsatisfactory. The lesioning was largely confined to the coleoptile with both these types of inoculation. Simmonds, Sallans, and Ledingham (26) demonstrated an abundance of conidia on wheat and barley straw and in surface soil. Lesions from this inoculum source developed at the ground level producing symptoms similar to those observed here.

The use of solid inoculum, consisting of the fungus and the medium employed for its growth, has long been a standard practice for the artificial induction of soil borne diseases (22). Satisfactory root disease was obtained in this work by infesting the planting soil with inocula produced by growing the fungus on various types of solid media. A standard medium consisting of sand, cornmeal, and nutrient salts was developed for use in the production of inoculum. This medium has several advantages over the other media tested. Its composition and method of preparation are easily standardized. Its loose texture allows a rapid and uniform growth of the fungus and it results in the production of a potent inoculum that can be easily and uniformly incorporated with the planting soil. Since completion of the work reported in this paper Butler (5) has described experiments on *H. sativum* and other root-rot organisms utilizing sand–cornmeal inoculum. Sallans (22) and Tyner (29) among others have pointed out that the addition of large amounts of organic matter to the soil was undesirable in that it was sometimes toxic. The amount of foreign organic matter added to the soil with the sand – cornmeal – nutrient salts inoculum is small since the medium itself contains less than 5% by weight of cornmeal. This is largely broken down by the fungus and is greatly diluted on addition to the planting soil. Some toxic effect does however result as evidenced by the depression of plant height shown in Fig. 12. This is contrary to the conclusion reached by Tyner (29) for a soil–cornmeal medium. The age of cultures used as inoculum is important since activity reaches a maximum and then declines. This occurred in 7 to 10 days under the growing conditions employed in experiments reported here. This is in general agreement with the results published by Tyner (29) and Sallans (22). The increase in activity with age provides further evidence of an effect of the organism distinct from any toxicity that might arise through the addition of organic matter.

A variety of methods have been used in the assessment of disease incidence. Christensen (6) and McKinney (19) proposed systems of numerical rating based on basal symptoms. Others have used shoot length and still others fresh and dry weights. A good correlation exists between these methods as shown by Broadfoot and Tyner (4) and confirmed in the present study. In dealing with artificially infected seedlings the use of plant height offers a rapid and objective method and is preferred by the authors. A numerical rating system however provides the only method for field assessment of disease.

The common method of artificial soil infestation for the induction of root disease has been by the addition of a single level of concentrated inoculum immediately beneath the seed at planting time (16, 22, 29). The practice of mixing inoculum uniformly with the planting medium has been followed less frequently (5, 13). The fallacies of the former procedure are apparent from the work reported in this paper. In the comparison of disease development in sand and greenhouse compost (see Fig. 3), for example, no differences are apparent in either the control plants or those grown at a high level of soil infestation, but the behavior in the two media is vastly different when the comparison is made over a range of infestation rates. The high level of soil infestation would give results comparable to those obtained when a layer of concentrated inoculum is placed beneath the seeds.

The necessity for the use of a series of dosages in evaluating the response of a living organism to a foreign agent is a well-established biological principle. Root disease studies of this type afford no exception.

Results show that a reasonable degree of reliability can be placed on comparisons made within an experiment when the experimental procedure is carefully standardized. Considerable caution must, however, be exercised in making such comparisons between experiments. This is illustrated in Fig. 2 where the 5% probability limits are shown for the average of a number of means for identical experiments. Much of this variability would undoubtedly be removed if the plants were grown under controlled environmental conditions.

The role of toxic substances produced by pathogenic fungi in the etiology of plant disease is becoming increasingly recognized. Dimond and Waggoner (9) have recently reviewed the literature bearing on this field hence a detailed consideration of it is unnecessary here. The occurrence of substances of biologic origin in soils that are detrimental to crop growth has long been recognized. Skinner and associates (27) noticed plant toxicity in leachates from a variety of soils. They isolated a number of aldehydes, notably salicylaldehyde and vanillin, in quantities sufficient to account for toxicity. It has been shown subsequently that apparently non-injurious amounts of salicylaldehyde will predispose sugar cane (21) and wheat (12) to *Pythium* root rots. Toxins produced by *Alternaria solani*, an organism having many characteristics in common with *H. sativum*, have been identified and studied

in relation to disease development (1, 2). Toxic products produced by *Gibberella fujikuroi* affect the development of rice seedlings and render them susceptible to attack by this organism (7, 28). Similarly toxin production by *Helminthosporium victoriae* and its role in disease development has been clearly demonstrated (15, 17, 30). Nothing has been reported, however, on the role of toxic substances in disease production by *H. sativum*, although a number of workers have seen evidence for such substances in their results (23, 22, 20). Shroeder (25) demonstrated a reduction in smut development in wheat as a result of inoculation with *H. sativum*. Experiments reported here establish toxin production by *H. sativum* and show that the substance (or substances) produced are essential for at least certain phases of disease production by this organism. Evidence showing that root invasion by other, normally non-pathogenic, microorganisms was promoted by the *Helminthosporium* toxin was also obtained. The manner in which the toxin predisposes the basal parts of the barley plant to fungal invasion and its role in the production of leaf spot and head blight symptoms is not clear. Investigations on these points are currently in progress.

Results reported in this paper indicate that the toxic substances produced by *H. sativum* are readily adsorbed in the soil and the degree of adsorption varies with different soils. A low incidence of disease, on artificial soil infestation, was associated with high toxin adsorption in the four soil samples examined. Evidence in the disease studies was against the conclusion that soil type differences are due to microbial antagonism although this possibility could not be entirely eliminated. *H. sativum* belongs to the group of soil fungi classified by Garrett (11) as root-inhabiting, and as pointed out by Simmonds and his colleagues (26), exists in the soil either as dormant spores or as spores and mycelium in crop debris. The latter can support good growth of the organism and could provide a reservoir of toxin that might have its effect when contacted by the germinating seed or growing plant.

Wheeler and Luke (30) recently described a method of utilizing the toxins of *H. victoriae* in selecting disease resistant oat seedlings. It is hoped that future studies will point the way to the development of a similar technique of value in the breeding of disease resistant barley and wheat varieties.

A more detailed understanding of the ecology and physiology of *H. sativum* is, however, necessary before a final understanding of the disease relationship can be reached.

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References

1. BRIAN, P. W., CURTIS, P. J., HEMMING, H. G., JEFFERYS, E. G., UNWIN, C. H., and WRIGHT, J. M. Alternaric acid, a biologically active metabolic product of *Alternaria solani* (Ell and Mart.) Jones and Grant; its production, isolation and antifungal properties. J. Gen. Microbiol. 5 : 619-632. 1951.
2. BRIAN, P. W., ELSON, G. W., HEMMING, H. G., and WRIGHT, J. M. The phytotoxic properties of alternaric acid in relation to the etiology of plant diseases caused by *Alternaria solani* (Ell and Mart.) Jones and Grant. Ann. Appl. Biol. 39 : 308-321. 1952.
3. BROADFOOT, W. C. Studies on foot and root rot of wheat. III. Effect of crop rotation and cultural practice on the development of foot rot of wheat. Can. J. Research, 10 : 95-114. 1934.
4. BROADFOOT, W. C. and TYNER, L. E. Studies on foot and root rot of wheat. VI. Methods of securing infection of wheat seedlings for study in nutrient solutions. Can. J. Research, C, 16 : 253-261. 1938.
5. BUTLER, F. C. Saprophytic behaviour of some cereal root-rot fungi. I. Saprophytic colonization of wheat straw. Ann. Appl. Biol. 40 : 284-297. 1953.
6. CHRISTENSEN, J. J. Studies on the parasitism of *Helminthosporium sativum*. Minn. Agr. Expt. Sta. Tech. Bull. 11. 1922.
7. CROSS, B. E. Gibberellic acid. Part I. J. Chem. Soc. 4670-4676. 1954.
8. DICKSON, J. G. Diseases of field crops. McGraw-Hill Book Company, Inc., New York. 1947.
9. DIMOND, A. E. and WAGGONER, P. E. On the nature and role of vivotoxins in plant disease. Phytopathology, 43 : 229-235. 1953.
10. FINNEY, D. J. Probit analysis. Cambridge University Press, London. 1952.
11. GARRETT, S. D. Ecology of the root-inhabiting fungi. Biol. Revs. Cambridge Phil. Soc. 25 : 220-254. 1950.
12. GRAHAM, V. E. and GREENBERG, L. The effect of salicylic aldehyde on the infection of wheat by *Pythium arrhenonenes* Drechsler, and the destruction of the aldehyde by *Actinomyces erythropolis* and *Penicillium* sp. Can. J. Research, C, 17 : 52-56. 1939.
13. GREANEY, F. J. and MACHACEK, J. E. Studies on the control of root-rot diseases of cereals caused by *Fusarium culmorum* (W. G. Sm.) Sacc. and *Helminthosporium sativum* P.K. and B. II. Pathogenicity of *Helminthosporium sativum* as influenced by *Cephalothecium roseum* Corda in greenhouse pot tests. Sci. Agr. 15 : 377-386. 1938.
14. LINN, M. B. and NEWHALL, A. G. Comparison of two methods of pelleting onion seed in the control of smut. Phytopathology, 38 : 218-221. 1948.
15. LITZENBERGER, S. C. Nature of susceptibility to *Helminthosporium victoriae* and resistance to *Puccinia coronata* in Victoria oats. Phytopathology, 39 : 300-318. 1949.
16. LUDWIG, R. A. and HENRY, A. W. Studies on the microbiology of recontaminated sterilized soil in relation to its infestation with *Ophiobolus graminis* Sacc. Can. J. Research, C, 21 : 343-350. 1943.
17. LUKE, H. H. and WHEELER, H. E. Toxin production by *Helminthosporium victoriae*. Phytopathology, 45 : 453-458. 1955.
18. MACHACEK, J. E. An estimate of loss in Manitoba from common root rot in wheat. Sci. Agr. 24 : 70-79. 1943.
19. MCKINNEY, H. H. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. J. Agr. Research, 26 : 195-217. 1923.
20. MEAD, H. W. Host–parasite relationships in a seed-borne disease of barley caused by *Helminthosporium sativum* P.K. and B. Can. J. Research, C, 20 : 501-523. 1942.
21. RANDS, R. D. and DOPP, E. The influence of certain harmful soil constituents on severity of Pythium root rot of sugar cane. J. Agr. Research, 56 : 53-68. 1938.
22. SALLANS, B. J. Methods of inoculation of wheat with *Helminthosporium sativum* P.K. and B. Sci. Agr. 13 : 513-527. 1933.
23. SALLANS, B. J. The use of water by wheat plants when inoculated with *Helminthosporium sativum*. Can. J. Research, C, 18 : 178-196. 1940.
24. SHERF, R. F., PAGE, R. M., TULLIS, E. C., and MORGAN, T. L. Studies on factors affecting the infectivity of *Helminthosporium oryzae*. Phytopathology, 37 : 281-290. 1947.
25. SHROEDER, H. W. Antagonistic action of *Helminthosporium sativum* and *Gibberella zeae* to infection by *Tilletia* spp. causing bunt of wheat. Phytopathology, 45 : 288. 1955.

26. SIMMONDS, P. M., SALLANS, B. J., and LEDINGHAM, R. J. The occurrence of *Helminthosporium sativum* in relation to primary infections in common root rot of wheat. Sci. Agr. 30 : 407-417. 1950.

27. SKINNER, J. J. Soil Aldehydes. A scientific study of a new class of soil constituents unfavourable to crops, their occurrence, properties and elimination in practical agriculture. J. Franklin Inst. 186 : 165-186, 289-316, 449-480, 557-584, 723-741. 1918.

28. TAMARI, K. and KAJI, J. Studies on the mechanism of the growth inhibitory action of fusarinic acid on plants. J. Biochem. (Japan), 41 : 143-165. 1954.

29. TYNER, L. E. Some factors affecting the virulence of artificial inoculum of *Helminthosporium sativum* P.K. and B. and *Fusarium culmorum* (W. G. Sm.) Sacc. Can. J. Research, C, 19 : 42-48. 1941.

30. WHEELER, H. E. and LUKE, H. H. Mass screening for disease resistant mutants in oats. Science, 122 : 1229. 1955.

STUDIES OF COPROPHILOUS ASCOMYCETES

II. PHAEOTRICHUM, A NEW CLEISTOCARPOUS GENUS IN A NEW FAMILY, AND ITS RELATIONSHIPS[1]

By Roy F. Cain[2]

Abstract

Phaeotrichum hystricinum Cain and Barr is described from specimens collected in Ontario, Vermont, New York, and Michigan and is made the type species of a new genus. It has been found on porcupine dung from numerous localities. The black, shining, superficial ascocarps are covered with scattered straight black appendages. The clavate stalked asci are in irregular fascicles, eight spored, and with a firm wall which is evanescent at maturity. The ascospores are two-celled, thick-walled, deeply constricted, and readily separating at the transverse septum. There is a large conspicuous germ pore at each end of the ascospore. *P. circinatum* Cain is described from specimens collected on lemming dung in Northern Ungava, Quebec. This species is distinguished from the former by means of the appendages, which are stouter and curved at the apex. These two species are closely related and very similar to the ostiolate *Trichodelitschia bisporula* (Crouan) Munk. The new genus is made the type of a new family of cleistocarpous Ascoloculares, with a discussion on the evolution, in many Ascomycete taxa, of cleistocarps adapted either to the utilization of special agencies, rather than air currents, for carrying the ascospores or to delayed dispersal.

Introduction

Two species of Ascomycetes of unusual structure have been found on specialized coprophilous habitats. One is widely distributed in Ontario and Michigan on the dung of porcupine; the other has been found only in the tundra and only on the dung of lemming.

In structure of the cleistothecial ascocarp, in arrangement of asci and their type, in the dark color of ascospores, and in coprophilous habitat, these two fungi bear some resemblance to *Perisporium vulgare* Corda and *P. funiculum* Preuss. But they differ from the latter at the family level by virtue of the possession of appendages on the ascocarp and in the structure of the ascospores, which are two-celled with a germ pore at each end rather than four-celled with elongated lateral germ slits as in the above-mentioned species of *Perisporium*. Therefore, we propose to place the two new species in a new genus, *Phaeotrichum*.

Since the two species of *Perisporium* mentioned above are not congeneric with the type of the genus (*P. speireum* Fries), it is necessary to determine which of several generic names available is the valid one for these species. An attempt is being made to do so, but meanwhile, it is clear that the family name, Perisporiaceae, is no longer available for any of these saprophytic fungi, including *P. vulgare* and *P. funiculum*. The structure of the ascospores indicates an entirely different origin from that of *Phaeotrichum*. Their

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relationship is remote enough to warrant placing them in separate families, although both are of the ascolocular type. They have practically nothing in common with members of the Aspergillaceae (Eurotiaceae) except the non-ostiolate character of the ascocarps, which, as will be shown below, is of little value in indicating relationships. I consider the Aspergillaceae, as well as the Erysiphaceae, to have been derived from taxa belonging to the ascohymenial rather than ascolocular series. The family Meliolaceae probably belongs with the Ascoloculares, but the parasitic habit and specialized type of mycelium exclude *Phaeotrichum*. It is consequently necessary to introduce a new family name.

The problem of ordinal relationship is a difficult one; it is discussed to some extent below, but, as will be seen, without arrival at a definite conclusion.

Descriptions

Phaeotrichaceae Cain fam. nov.

Saprophytic; ascocarps unilocular, stromatic, superficial, cleistocarpous. Asci in irregular or parallel fascicles, evanescent at maturity. Ascospores dark-brown, with terminal germ pores.

Saprophyticae; ascocarpi superficiales, uniloculares, stromaticii, sine ostiolo. Asci sive parallele sive irreguliter in fasciculis dispositi, evanescentes. Ascospori atro-fusci.

Type genus. *Phaeotrichum* (*phaios*, φαιοσ, dusky and *thrix*, θριξ, hair).

Phaeotrichum Cain and Barr gen. nov.

Saprophytic, coprophilous. Ascocarps superficial, cleistocarpous, unilocular, stromatic, thin-walled, black, shining, globose, carbonaceous–membranaceous, with scattered black appendages. Outer covering of ascocarp consisting of thick-walled, dark-brown, angular cells. Asci eight-spored, broadly clavate, stipitate, without pore or thickening in apex and without special means of dehiscence. Paraphyses absent. Ascospores in several series, deeply constricted at the single median transverse septum, dark-brown and opaque with thick wall and two circular germ pores, one at each of the broadly rounded ends. Cells of ascospores ellipsoid, readily separable at maturity.

Saprophyticae; fimicolae; ascocarpi superficiales, globosi, uniloculares, stromaticii, carbonaceo–membranacei, nigri, cum setulis longis atris vestiti, non ostiolati. Asci late clavati, stipitati, octospori, evanescentes; aparaphysati. Ascosporae oblongo-ellipsoideae, biloculares, atro-fuscae, profunde constrictae, in medio facillime secedentes.

Type species. *Phaeotrichum hystricinum* Cain and Barr (*hystrix*, porcupine).

Key to Species

Appendages on ascocarp long and straight or slightly flexed, about 3 μ wide near apex..... 1. *P. hystricinum*

Appendages broader, circinate at outer end..... 2. *P. circinatum*

1. Phaeotrichum hystricinum Cain and Barr sp. nov. Figs. 1–11

Ascocarps scattered or loosely aggregated, superficial, globose, opening by an irregular break in the wall, 150–200 μ in diameter (rarely up to 300 μ), black, shining, with 15–20 long straight, or sometimes slightly flexed, black appendages uniformly scattered over entire surface. Appendages 500–1000 μ in length (mostly 500–600 μ), 20–25 μ wide at the base, abruptly tapered to about 7 μ and then gradually tapered to about 3 μ near blunt apex; appendages usually non-septate but rarely with two or three septa near the base, with wall about 1.0 μ in thickness, very black and opaque. Outer peridium of ascocarp thin, carbonaceous–membranaceous, composed of usually one layer of thick-walled (0.5–1.5 μ), dark-brown, angular cells measuring 8–16 μ in diameter, not arranged in plates, without special cleavage areas, with a somewhat radiate arrangement at base of hair-like appendages. Asci eight-spored, broadly clavate, 42–48 × 14–17 μ (spore bearing portion 22–30 × 14–17 μ), broadly rounded above, abruptly narrowed below the spores to an elongated stipe about 18 μ in length, forming irregular fascicles, with a firm wall which is evanescent at maturity and without apical pore or thickening. Paraphyses absent. Ascospores crowded in many series in upper swollen part of ascus, oblong-ellipsoid, 13.5–15.5 × 4.0–5.0 μ, two-celled with a broad deep constriction at the single transverse thick septum, each end of ascospore provided with a broad subhyaline thin circular germ pore, the smooth ascospore wall otherwise thick, hyaline at first, then yellow and finally very dark brown and opaque at maturity. The two cells of the ascospore individually ellipsoid, readily separable when mature.

Ascocarpis sparsis vel subdense gregariis, superficialibus, globosis, non ostiolatis, 150–200 (300) μ, nigris, politis, setulis atris, opacis, rectis, aequabiliter dispersis, 500–1000 μ (plerumque 500–600 μ) longis, basi 20–25 μ, medeo 7 μ, apice 3 μ latis, obtusis, non septatis, rare 2–3-septatis vestitis. Peridio tenui, carbonaceo–membranaceo, e cellulis atro-brunneis, angulatis, 8–16 μ constituto. Ascis octosporis, late clavatis, 42–48 × 14–17 μ, superne subtruncatis, basin versus in stipitem 18 μ longam abrupte attenuatis, sive parallele sive irregulariter in fasciculis dispositis, ad apicem non perforatis, necnon incrassatis, evanescentibus, sine paraphysibus. Ascosporis multiseriatis in parte superiore ascorum dense aggregatis, oblongo-ellipsoideis, 13.5–15.5 × 4.0–5.0 μ, bicellulis, ad septum unicum transverum crassum profunde constrictis, facile secedentibus. Episporio crasso, laevi, primo hyalino, deinde luteo, demum atro-brunneo opacoque, utrinque cum foramine orbiculato ornato.

Collections

All on porcupine dung.

Ontario: Muskoka Dist., Stoneleigh, Aug. 18, 1932 (**Type** TRTC 4361). Lake Timagami, Bear Island, Sept. 11, 1935. Aug. 5, 1937. South Arm, Aug. 11, 1931. Cross Lake Portage, Aug. 28, 1931. Kokoko Bay, July 12, 1931. Aug. 16, 1931. Gull Lake Portage, Sept. 9, 1935. Aug. 20, 1936. Peel

County, Mono Mills, June 21, 1947. (TRTC 31621-31632). (The following numbers are collections developed in moist chamber in laboratory: 31621, 31625, 31628, 31630, 31632, 31633.) All collected by R.F.C.

New York: Adirondacks, Seventh Lake, Aug. 21, 1934 (TRTC 31633), collected by R.F.C.

Vermont: Mt. Mansfield, June 18, 1951 (TRTC 31620), collected by R.F.C.

Michigan: Mackinac Co., St. Ignace, Aug. 7, 1953, collected by M. E. Barr (1277). Cheboygan Co., The Gorge, University of Michigan Biological Station, Aug. 21, 1953, collected by M. E. Barr (1483).

2. *Phaeotrichum circinatum* Cain sp. nov. Figs. 12–19

Ascocarps scattered or loosely aggregated, superficial, globose, opening by an irregular break in the wall, 150–230 μ in diameter, black, shining, with about 10–25 circinately-tipped appendages uniformly scattered over entire surface. Appendages measuring 150–200 μ from base to outer part of arch, mostly non-septate, rarely with two or three septa near base, 15–25 μ wide at base, abruptly tapered to 7–9 μ then gradually tapered to 4–7 μ at the circinate apex, very blunt at tip, with wall very thick (about 2 μ), black and opaque. Outer peridium of ascocarp thin, carbonaceous–membranaceous, composed of about one layer of thick-walled, brown, angular cells measuring 6–10 μ in diameter, not arranged in plates, without special cleavage areas, with a somewhat radiate arrangement at base of appendages. Wall of cells irregularly thickened with small darker spots. Asci eight-spored, broadly clavate, 35–45 $\times$ 15–18 μ, broadly rounded above, abruptly narrowed below the spores to an elongated stipe about 15 μ in length, forming irregular fascicles as side branches from irregular chains of cubical ascogenous cells, with a firm wall in immature stages, evanescent at maturity and without apical pore or thickening. Paraphyses absent. Ascospores crowded in many series in upper swollen part of ascus, oblong-ellipsoid, 11–15 $\times$ 4.0–4.8 μ, two-celled with a broad deep constriction at the single transverse thick septum, each end of ascospore provided with a broad subhyaline thin circular germ pore, the smooth ascospore wall otherwise thick, hyaline at first, then yellow and finally very dark brown and opaque at maturity. The two cells of ascospore individually ellipsoid, readily separable when mature.

Ascocarpis sparsis vel subdense gregariis, superficialibus, globosis, non ostiolatis, 150–230 μ, nigris, politis, setulis atris, opacis, ad apicem circinatis, aequabiliter dispersis, 150–200 $\times$ 4–9 μ, obtusis, non septatis (interdumque 2–3-septatis observatis) vestitis. Peridio tenui, carbonaceo–membranaceo e cellulis atro-brunneis, angulatis, 6–10 μ constituto. Ascis octosporis, late clavatis, 35–45 $\times$ 15–18 μ, superne subtruncatis, basin versus in stipitem 18 μ longam abrupte attenuatis, sive parallele sive irregulariter in fasciculis dispositis, ad apicem non perforatis, necnon incrassatis, evanescentibus, sine paraphysibus. Ascosporis multiseriatis in parte superiore ascorum dense aggregatis, oblongo-ellipsoideis, 11–15 $\times$ 4.0–4.8 μ, bicellulis, ad septum unicum, transversum, crassum profunde constrictis, facile secedentibus.

Episporio crasso, laevi, primo hyalino, deinde luteo, demum atro-brunneo opacoque, utrinque cum foramine orbiculato ornato.

Collections

All on lemming dung.

Quebec: Ungava, Payne River at outlet of Payne Lake, 73° 27′ W. long. Aug. 5, 1948 (**Type** TRTC 31634). First Fall of Mogaluk, 20 miles from Hudson Bay, July 20, 1948, developed in moist chamber in laboratory at Toronto, June 1949 (TRTC 31635). All collected by J. Rousseau.

Relationships

P. hystricinum and *P. circinatum* are very similar except for the appendages. In the former they are straight or only slightly flexed and considerably tapered from the base to near the apex. In the latter they are only slightly narrowed toward the apex with a broad circinate tip which probably assists in ascocarp dispersal.

The wall of the hair-like appendages in both of these species is unusual in its dense and uniform pigmentation. This is very much like the wall of the spiny hairs present on the ostiolate ascocarps of *Trichodelitschia bisporula* (Crouan) Munk (3). There is reason to believe that the species of *Phaeotrichum* have evolved from a taxon fairly close to *T. bisporula*. The hairs are still spine-like but have become much elongated in *P. hystricinum* as an aid to ascocarp dispersal, probably by small invertebrates inhabiting the dung or on the surface of the porcupines. This species, although widely distributed, has been found only on the dung of porcupine and more frequently on the heaps in the dens of the animals than on the dung pellets scattered on the ground. The porcupine feeds on bark rather than herbaceous plants. *T. bisporula* (Fig. 20) is found on the dung of herbivorous animals, especially rabbits, and as an adaptation to this habitat the ascospores have a gelatinous sheath by means of which they stick to the surface of vegetation which may be eaten by the animals. In *Phaeotrichum* there is no gelatinous sheath around the spores. In a future publication I shall describe a species of *Sordaria* that inhabits the dung of a carnivore and in which the ascospores have no gelatinous sheaths, but which is closely related to a species of this genus that possesses a sheath and inhabits the dung of herbivorous animals.

In *P. circinatum* the appendages are broader, have a more uniform diameter, and are circinate at the tip. These probably hook on to the hair of the lemming and thus the ascocarps may be carried to a new location. The fungus has been found only on the dung of lemmings. It is of interest to note that the appendages become distinctly circinate only as the ascocarp reaches maturity.

Except for the smaller size, deeper constriction, and lack of gelatinous sheath, the ascospores of the two species of *Phaeotrichum* are very similar to those of *Trichodelitschia bisporula*. The latter genus was established by Munk (3) for *Delitschia bisporula*, a species with opaque, spiny hairs and

ascospores containing a circular germ pore at each end. Typical species of *Delitschia* have a lateral germ slit extending the length of each cell and have no spiny hairs. The asci of *T. bisporula* are the bitunicate type as defined by Luttrell (2) and in this respect are similar to species of *Delitschia*. In both genera the asci are arranged in a parallel fascicle. *Phaeotrichum* has broadly clavate asci in contrast to the cylindrical ones of *Trichodelitschia*. In the former genus the asci do not elongate just prior to ascospore discharge but break down at maturity.

The bitunicate ascus is a very characteristic feature of the Ascoloculares. The outer non-living wall is rigid at maturity and does not stretch. When the pressure of the living plasma membrane within becomes too great the outer wall breaks some distance below the apex so that a thimble-shaped upper end is pushed forward with the elongating inner membrane so that the ascus rapidly extends to about double its length.

The terms bitunicate and unitunicate are somewhat misleading since in both types the ascus has a single non-living wall on the outside and a single living plasma membrane within. The real distinction of the bitunicate ascus lies in the structure of the outer wall, which is such that it is non-elastic at maturity in contrast to that of the unitunicate, which stretches to allow the enlargement of the ascus prior to spore discharge.

In the bitunicate type of ascus, both the outer wall and inner membrane are usually thicker than the same structures in the unitunicate type. But among those fungi with the latter type are many species of Inoperculate Discomycetes and Disco-lichens with a very thick wall and membrane, especially those adapted to xerophytic conditions.

In *Phaeotrichum* there is no ostiole in the ascocarp and the ascospores are no longer discharged for aerial dispersal. The asci do not elongate prior to forcible ascospore discharge but simply break down and allow the contents to become free in the cavity of the ascocarp. The ascogenous hyphae ramify in a somewhat irregular manner through a large part of the internal part of the ascocarp. The asci still show some of their ancestral fascicled arrangement, but are no longer in a single parallel layer and as a result are broader but still have a distinct stipe. In their shape and arrangement they have retained some of the characteristics of the bitunicate ascus. But they have lost the ability to elongate and do not discharge the ascospores through an apical pore.

It is becoming increasingly evident that the taxa Plectascales and saprophytic Perisporiales do not represent natural orders or even a few related groups but a miscellaneous assemblage of a large number of phylogenetically unrelated taxa in which the ascocarps produce ascospores no longer adapted to aerial dispersal by being forcibly discharged into the atmosphere.

In my opinion, random wind dispersal of ascospores is basic throughout the Ascomycetes, the mechanisms of adaption to more specialized means representing a more recent evolution.

In some cases specialization involves a delayed dispersal. Ascocarps of this type are found in fungi adapted to living in ephemeral substrata. They mature

rapidly and then the ascospores are gradually disseminated. *Chaetomium* species are typical examples of this type. The ascocarps still produce an ostiole, but the asci break down at maturity and the ascospores are retained in a mass by means of the perithecial hairs, which are usually branched or coiled. The closed ascocarps may carry the fungus over unfavorable seasons, such as winter in the case of members of the Erysiphales, or dry periods. The Ascocarps of species of *Aspergillus* and *Penicillium* remain viable when dried for many years.

It is of interest to note that a secondary development in many Basidiomycetes such as *Lycoperdon* has evolved a basidiocarp adapted to delayed dispersal of the basidiospores.

A second type of modification away from the immediate aerial dispersal of ascospores has resulted in the production of a form of ascocarp adapted to areas not directly exposed to free air currents. This type is found in soil, humus, and masses of rapidly decaying vegetation. *Emericellopsis* and *Thielavia* are typical examples among the Plectascales. Most of the Tuberales are of this type as well as the Melanogastraceae, which serves as an example among the Basidiomycetes.

A still further type of modification in the dispersal mechanism has resulted from adaptation to the use of invertebrate animals such as insects for ascospore dissemination. This is particularly common in species of fungi inhabiting special types of environment. This method is much less wasteful of ascospores than is random wind dispersal. The ascocarps of the fungi are especially adapted in some cases by having a fringe of hairs at the apex of a long neck which holds a viscid mass of ascospores resulting from the breakdown of the asci at maturity. Species of *Ceratocystis*, *Melanospora*, and *Sphaeronaemella* are examples of this type. Species of fungi with cleistothecial ascocarps have evolved special appendages as an adaptation to transportation by animals. Examples of this type are found in the genera *Phaeotrichum*, *Magnusia*, *Gymnoascus*, and *Ctenomyces*. Some species of *Chaetomium* such as *C. circinatum* and *C. murorum* have circinate appendages which serve much the same purpose.

A somewhat different type of ascocarp adapted to either delayed dispersal or invertebrate carriers is found in the lichen families Sphaerophoraceae and Caliciaceae. The ascospores accumulate as a powdery mass as a result of the breakdown of the asci. This structure is called a mazaedium. The ascocarps of species of *Onygena* show a somewhat similar type of modification. A different, but as yet unidentified, fungus found growing on a twig on the ground produces a stalked head of asci which break down to form a powdery mass of ascospores.

Examples of Basidiomycetes showing a similar modification adapted to dispersal of spores by means of invertebrates instead of random wind dissemination are found in the families Clathraceae and Phallaceae.

In the Ascomycetes, the basic forms produce ascocarps in which the asci are oriented in such a way and so constructed that they can forcibly expel

their spores and launch them on air currents. An unenclosed hymenial layer or the presence of a structural ostiole within reach of the mature asci provide the possibility for such launching. But when wind is not the agent of dispersal, modification can occur in the spatial relationship of ostiole to asci, in the structure of the ostiole even to the point of its elimination, in the structure of the asci and their orientation, in the reduction of auxiliaries no longer needed for such orientation, or the development of other auxiliaries serving new functions, and so on.

The cleistothecial type of ascocarp is the result of a progressive evolution away from dispersal of ascospores by means of air currents immediately following their production. It has evolved at many different points from various taxa of Ascomycetes with more elaborate types of ascocarps from which the ascospores are forcibly discharged by the asci. The presence of a so-called cleistothecium does not of itself indicate relationship at any level including order, family, or genus. Doguet (1) has shown that some species of *Melanospora* which normally produce an ostiole may, under cultural conditions, form a cleistothecium. Species of *Chaetomidium* which are characterized by having a cleistothecium are very close to species of *Chaetomium* whose ascocarps develop ostioles. Fairly close connections can be demonstrated between species with cleistothecia and taxa typical of the families Pezizaceae, Nectriaceae, Sphaeriaceae, and Sordariaceae and families of the Ascoloculares. The details of these connections will be left to future publications.

Along with the evolution of the cleistothecial type of ascocarp there has been a modification of the ascus arrangement. In ascocarps where the ascospores are forcibly discharged the asci are in a single more or less parallel layer. There has been a progressive evolution away from this arrangement accompanied by the production of shorter asci. In *Phaeotrichum* the asci are broadly clavate and in irregular fascicles so that this genus occupies an intermediate position with respect to this feature. In lines where the evolution has progressed further there is a more extensive development of ascogenous hyphae with the formation of numerous globose asci on an irregular branching system.

The modification from the palisade arrangement of the asci toward an irregular orientation has taken place in many different lines and is an indication of the level to which evolution has progressed. The development of asci in various directions within the ascocarp taken by itself does not always indicate relationship. The globose type of ascus has evolved from the elongate type in many different lines and taken by itself is not an indication of relationship.

Paraphyses are lacking in nearly all species with cleistocarps and when present are very rudimentary. It would appear that they are no longer functional in maintaining the orientation of the asci in the direction of the opening of the ascocarp or in keeping the hymenium turgid with varying numbers of maturing asci. Production of paraphyses no longer takes place in

a number of evolutionary lines of ostiolate species. Some of these involve very small ascocarps, as in species of *Mycosphaerella*, where paraphyses are apparently no longer needed. In other ascocarps where paraphyses are lacking the asci break loose and are pushed out of the ostiole or break down while still inside. The absence of paraphyses, by itself, is of little value in determining relationships.

The location of the paraphyses within the ascocarp has been used as a means of delimiting the Ascoloculares. In this taxon they usually originate in the upper region of the ascocarp and have been called pseudoparaphyses. The value of this distinction as an indication of relationship is open to question. In some of the Hypocreales and the closely related *Chaetomium* the paraphyses originate largely from the upper part of the ascocarp. In the Patellariaceae, which belong in the Ascoloculares, the paraphyses and asci both originate in the subhymenial region and have their free ends oriented in the same direction. The same is true of some lichen genera which have the ascolocular type of structure.

In summary it may be said that of all the characters used to delimit the Plectascales none are of any great significance in so far as indicating relationships is concerned and that the order is not a natural one. The species included have very diverse affinities and have a mere superficial resemblance. Real relationship can be determined only by detailed study and comparison of the fungi themselves, their complete morphology (including conidia and the manner in which they are produced), as well as all stages of development of the ascocarps. The physiology and chemical reactions of the fungi concerned should also be taken into consideration. Correlation of several characteristics is more useful in a natural delimitation of taxa be it order, family, or genus. This is especially true in the Ascomycetes. The presence of a dark-brown pigment is very characteristictic of the Ascoloculares but taken by itself cannot always be relied upon as a key character. A tendency to produce septate ascospores is a significant feature of the same taxa but is by no means limited to this group. A combination of dark-brown pigment and many-septate ascospores is much more likely to indicate one of the Ascoloculares. The bitunicate ascus is produced by most of these fungi, but the lack of this characteristic ascus does not, taken by itself, exclude a fungus from close relationship.

It is self-evident that as long as the mycologist avoids tackling the problem of the distinction between parallel evolution and homologous development, his classification of the fungi will remain purely artificial. During the past twenty years of study, it has become apparent to me, as to many others, that many of the characteristics which we take to be diagnostic of individual phylogenetic lines are better interpreted as evolutionary levels potential or already developed in quite different lines, either adaptations to the environment in which their members find themselves, or features correlated with such adaptations.

References

1. DOGUET, G. Le genre *Melanospora*, biologie, morphologie, développement, systématique. Botaniste, 39 : 1-313. 1955.
2. LUTTRELL, E. S. Taxonomy of the Pyrenomycetes. Univ. Missouri Studies, 24 (3) : 1-120. 1951.
3. MUNK, A. The system of the Pyrenomycetes. Dansk botan. Arkiv. 15 : 1-163. 1953.

EXPLANATION OF FIGURES

FIGS. 1–11. *Phaeotrichum hystricinum.*

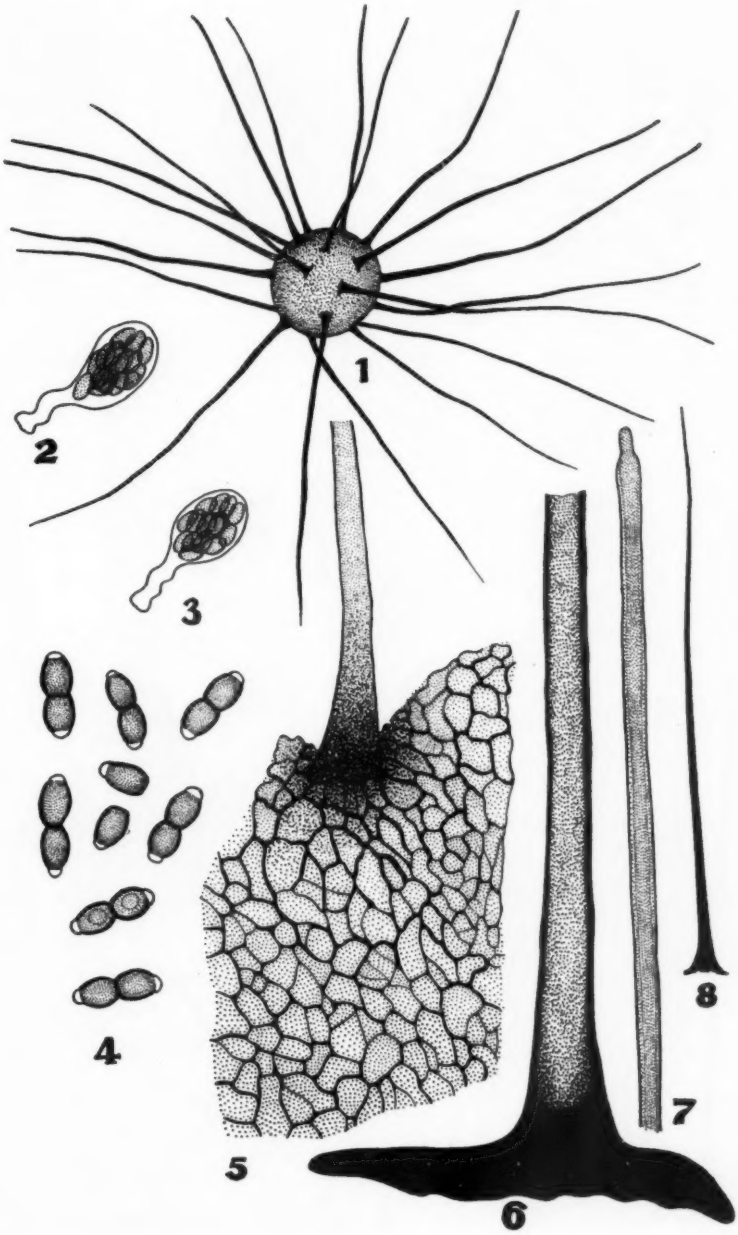
FIG. 1. Ascocarp with appendages. × 80. FIGS. 2, 3. Asci. × 520. FIG. 4. Ascospores. × 900. FIG. 5. Part of peridium of ascocarp with base of an appendage. × 550. FIG. 6. Base of appendage. × 900. FIG. 7. Apex of appendage. × 900. FIG. 8. Appendage. × 150. FIG. 9. Part of peridium with base of an appendage. × 1270. FIG. 10. Two asci. × 1270. FIG. 11. Ascospores. × 1270.

FIGS. 12–19. *Phaeotrichum circinatum.*

FIG. 12. Young asci and ascogenous cells. × 1140. FIG. 13. Two asci. × 1140. FIG. 14. Ascospores. × 1140. FIG. 15. Ascocarp with appendages. × 110. FIGS. 16, 18. Appendages. × 600. FIG. 17. Peridial cells of ascocarp with appendage. × 500. FIG. 19. Appendage. × 1140.

FIG. 20. *Trichodeitlschia bisporula.* Ascus with ascospores. × 1140.

NOTE: FIGS. 1–20 follow.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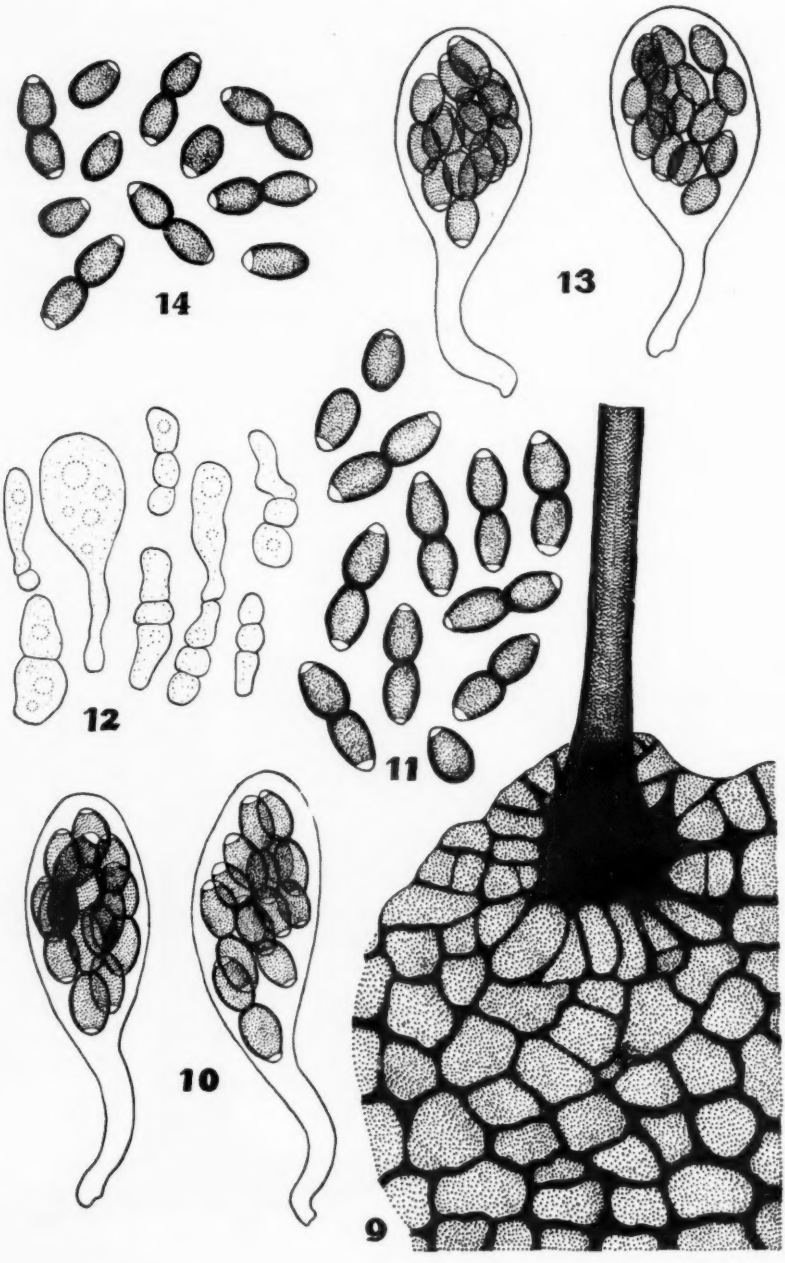
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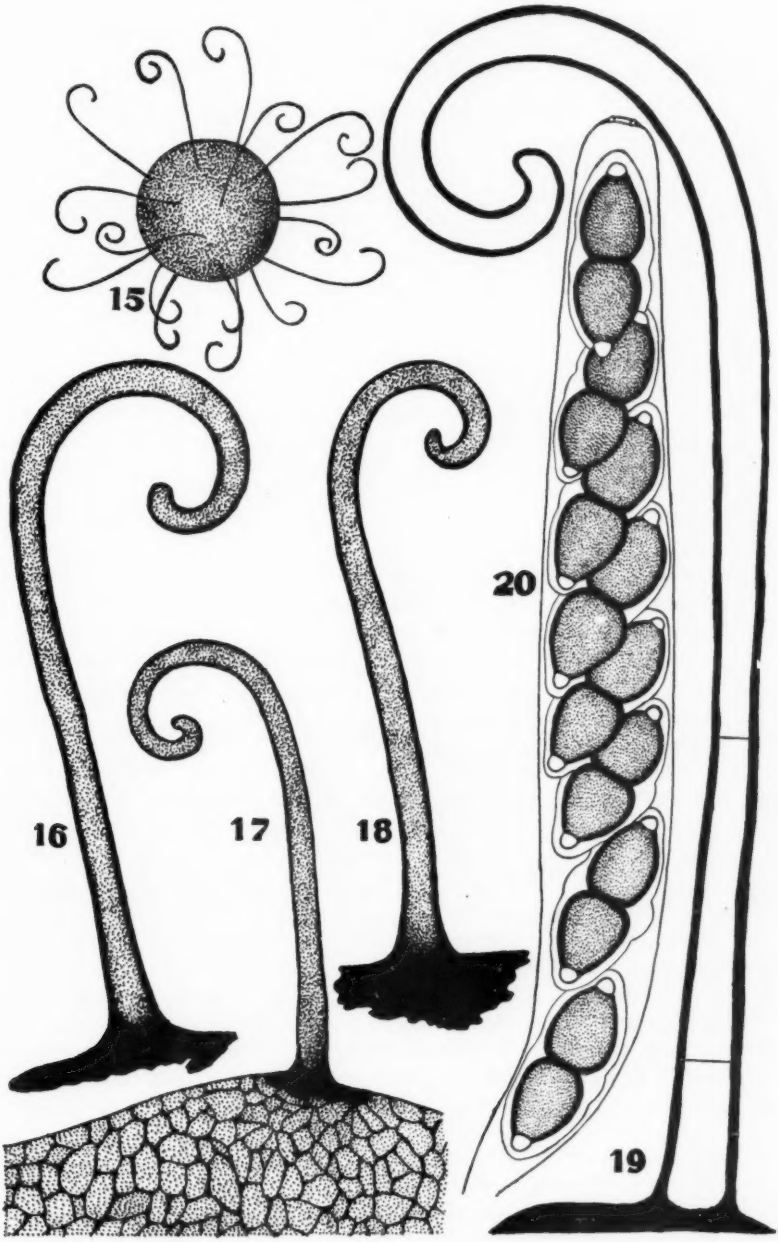
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STUDIES OF COPROPHILOUS ASCOMYCETES

III. THE GENUS TRIANGULARIA[1]

By Roy F. Cain[2] and W. M. Farrow[3]

Abstract

Two new species are added to the monotypic genus *Triangularia*. The first, *T. angulospora*, was found on dung and soil from the Panama Canal Zone; the second, *T. obliqua*, was obtained from an African collection of the dung of Liberian antelope. The three species of *Triangularia* are considered to have evolved from species of *Podospora* (*Sordaria*) from which they are distinguished by the character of their ascospores. These are angular, usually inequilateral in shape, with a short, broad, hyaline basal cell or primary appendage, the secondary gelatinous appendages absent or much reduced.

Introduction

In 1934 Boedijn (3) proposed the generic name *Triangularia* for *Trigonia bambusae* van Beyma (2) since the name *Trigonia* Aubl. had been used previously for a genus of phanerogamic plants. The characteristic feature of the only species known is the triangular shape of the ascospores. These are elliptical when young, but soon become flattened on one side because of inequilateral development. The ends are rather sharply angled and in addition a third angle is formed on the convex side. A septum parallel to the side of the ascospore opposite to the summit of the ascus cuts off a disk-shaped cell which remains hyaline. The larger upper cell lays down a dark-brown wall so that the spores appear black by reflected light.

The structure of the perithecia and asci is typical of *Podospora* (*Pleurage*, *Sordaria*) to which *Triangularia* is evidently related as has been pointed out by C. Moreau (5) as well as M. and Mme F. Moreau (6). Van Beyma (2) suggested a relationship between *Trigonia* and *Delitschia*, the latter distinguished by means of ascospores which have two dark cells. However, he overlooked the fact that the structure of the perithecia, asci, and ascospores of *Delitschia* is of the Ascoloculares type.

Triangularia has remained monotypic. If its only distinction were the fact that the ascospores are triangular it would be questionable whether it would be worthy of maintenance as a genus separate from *Podospora* (*Sordaria*). A number of genera are known in which some species have triangular ascospores and some ellipsoid ones. *Chaetomium trigonosporum* (Marchal) Chivers has ascospores which are triangular in shape in one view. Among the appendaged species of *Sordaria* (*Podospora*) there are several species with inequilateral development of the ascospores, for example: *S. fimbriata* Bayer, *S. inaequilateralis* Cain, *S. inaequalis* Cain, and *S. curvispora* Cain. Inequilateral

[1]*Manuscript received April 18, 1956.*
Contribution from the Department of Botany, University of Toronto, Toronto, Canada. This study was carried out with the assistance of a grant in aid of research to the senior author, furnished by the University of Toronto.
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ascospores are also known in the genus *Melanospora*. There is, however, a more significant feature of the genus *Triangularia* in the development of the ascospores. These are at first elliptical rather than pyriform as in most species of *Podospora*. Consequently, the septum which cuts off the hyaline basal cell is a broad one. Two additional species (*T. obliqua* and *T. angulospora*) described in the present paper have a similar broad septum. In addition, there are several other features common to the three species. The ascospores are all inequilateral or angular, the hyaline basal cell has a broad base, and the gelatinous appendages of the ascospores are either absent or very small and inconspicuous.

Key to the Species of *Triangularia*

Septum in ascospores transverse, with dark upper cell five-angled (in side view)..... ..... 1. *T. angulospora*

Septum in ascospores oblique
- In side view, ascospores triangular, with triangular dark upper cell..... 3. *T. bambusae*
- In side view, ascospores with subovate, slightly oblique upper dark cell, and oblique hyaline cell..... 2. *T. obliqua*

1. Triangularia angulospora Cain and Farrow sp. nov. Figs. 1–3, 10–15

Mycelium spreading rapidly in culture, white, producing a loose layer with little aerial growth. Perithecia brownish-black, superficial or with base slightly immersed, dispersed, or aggregated in small clusters, 300–460 × 200–300 μ, pyriform, constricted above to form a short, stout, papilliform neck with a circular ostiole, 50 μ in diameter at apex. Perithecial peridium dark-brown, membranaceous, consisting of rounded to somewhat angular cells, upper part and neck darkened by irregular patches of minute tubercles. Hairs straight, septate, brown, 50–100 × 3.5–4.0 μ, blunt at apex, scattered over neck and upper part of perithecium. Asci eight-spored, narrowly clavate, broadly rounded above, with distinct thickened ring in apex, tapering gradually below into a short stipe, 100–150 × 16–19 μ. Paraphyses filamentous, septate. Ascospores biseriate above, uniseriate below, at first hyaline and ovate-elliptical, becoming two-celled by the formation of a transverse septum; upper cell changing through olivaceous-brown to dark-brown and opaque, rather sharply angled at broadest part and pointed at apex so as to appear five-angled in side view, 13–15 × 9–12 μ, with a single, circular, subapical germ pore measuring 1 μ in diameter, and with a small gelatinous appendage measuring 3 μ long and 2 μ wide at apex; lower cell remaining hyaline, becoming devoid of protoplasm, 5–7 μ long, 7–8 μ wide at base, narrowed toward the broadly rounded end, usually slightly oblique. Phialides scattered on the mycelium, rarely produced, up to 15 μ long, rather indistinct. Phialospores hyaline, ovate, 3–4 × 2.5–3.0 μ, accumulating in small heads.

Isolated in pure culture, December 1952, by W. M. Farrow from soil collected on Barro Colorado Island, Panama Canal Zone, July 1952, by G. W. Martin and A. L. Welden. **Type.** IA, Martin 8746, TRTC 31639. On horse dung, Gatun, Panama Canal Zone, R. Thaxter FH, TRTC 31638.

Mycelio niveo, tenue, propere aucto. Peritheciis dispersis vel in caespites aggregatis, 300–460 × 200–300 μ, superficialibus vel basi partim immersis, piriformibus, supra in collum brevum crassum papilliforme attenuatis, ostiolis 50 μ crassis ad apicem praeditis; parietibus atro-brunneis, membranaceis, in parte superiore cum tuberculis in centonibus et cum pilis rectis, septatis, brunneis, 50–100 × 3.5–4.0 μ, ad apicem obtusis vestitis. Ascis octosporis, tenuiter clavatis, 100–150 × 16–19 μ, superne late rotundatis, ad apicem orbibus praeditis, basi in stipitem brevem attenuatis. Paraphysibus filiformibus, septatis. Ascosporis superne biseriatis, inferne uniseriatis, initio hyalinis, ovato-ellipticis, non septatis, dein transverse uniseptatis. Cellula superiore, in maturitate atro-brunnea opacaque, ad partem latissimam acriter angulatam, ad apicem acuminata, quinangularia in conspectum venta, 13–15 × 9–12 μ, foramine germinali, singulari, orbiculato, subapicali, 1 μ crasso praedita et ad apicem appendice gelatinosa, 3 μ longa, 2 μ lata ornata. Cellula inferiore hyalina, 5–7 μ longa, ad basem 7–8 μ lata, plerumque leniter obliqua. Phialidibus solis, usque 15 μ longis, raris. Phialosporis hyalinis, ovatis, 3–4 × 2.5–3.0 μ, incapitula coacervatis.

T. angulospora was designated "unknown No. 1" by Farrow (4).

The production of perithecia is very erratic in culture. They were obtained by the junior author on corn stalks or oatmeal flakes in a basal medium consisting of the following constituents: NH_4NO_3, 3.0 gm.; KH_2PO_4, 1.0 gm.; $MgSO_4 \cdot 7H_2O$, 0.5 gm.; KCl, 0.5 gm.; agar, 15.0 gm.; distilled water, 1 liter. Perithecia matured in about eight to eleven days.

The first culture received by the senior author failed to produce perithecia on a modified Leonian's agar. A second culture was obtained at a later date. This was on corn stalks plus basal medium and had mature perithecia from which the ascospores were forcibly discharged. When transfers were made to corn stalks or oatmeal flakes in basal medium at Toronto no perithecia were formed. Transfers from the same culture to a modified Leonian's agar produced abundant perithecia with normal ascospore discharge. The modified Leonian's agar was made as follows: KH_2PO_4, 1.2 gm.; $MgSO_4 \cdot 7H_2O$, 0.6 gm.; peptone, 1.0 gm.; maltose, 6.0 gm.; malt extract, 6.0 gm.; yeast extract, 3.0 gm.; agar, 20 gm.; water, 1 liter.

In many of the cultures of *T. angulospora* there is a tendency for the ascospores to accumulate in a mass at the ostiole of the perithecium, because the asci are unable to forcibly discharge their contents into the air. This is probably due to a film of water over the ostioles caused by the moist conditions in the culture tubes, and not to any failure in the expulsion mechanism of the ascus. A similar tendency toward a failure in forcible ascospore discharge and production of a mass of ascospores at the ostiole was noted by Moreau and Moreau (6) in cultures of *Triangularia bambusae*. However, in many of the test tube cultures of *T. angulospora* as well as those of *T. bambusae*, there was a normal black deposit of ascospores on the side of the tube opposite to and above the perithecia.

The accumulation of a mass of ascospores at the ostiole is a common phenomenon in species belonging to many related genera, especially when these are kept in culture or moist chamber. It has been observed by the senior author in species of *Sordaria*, *Podospora*, *Nectria*, *Hypomyces*, and *Hypocrea*. This characteristic is considered to be of no very great taxonomic value.

2. *Triangularia obliqua* Cain. Figs. 4–9, 16–18

Perithecia ovate to pyriform, black, superficial, numerous, and evenly spaced over the substratum, 200–230 × 120–150 μ, narrowed above into a short broad neck which is covered with hairs. Perithecial peridium dark-brown by transmitted light, semitransparent, membranaceous. Peridial cells rather obscure, small, irregular, 6–12 μ in diameter. Hairs 50–100 × 3–4 μ, straight or slightly flexuous with firm wall, brown, lighter in color toward tip, blunt at apex, scattered over upper part of perithecium. Asci eight-spored, clavate, broadly rounded above, with thickened ring in apex, tapering gradually below into a stipe, 165–190 × 30–35 μ. Paraphyses filiform, septate. Ascospores biseriate, at first hyaline, ovate-elliptical, slightly narrowed toward upper end, more conspicuously narrowed toward lower end, becoming inequilateral with the lower end bent to one side, becoming two-celled by the formation of an oblique septum so oriented that the part of the septum nearest the flattened side of spore is directed toward the base of ascus; upper cell (nearest apex of ascus) becoming dark brownish-black at maturity, 19–23 × 12–14 μ, with a circular, apical, nearly hyaline germ pore measuring 2 μ in diameter and a hyaline, gelatinous, curved, indistinct, fugaceous, secondary appendage measuring 10 μ long and 2 μ wide, attached slightly below the apex on the more flattened side of spore; lower cell of ascospore remaining hyaline (or sometimes light-brown), finally devoid of visible contents, oblique, curved toward more flattened side of ascospore, measuring 10–12 μ along the septum, with a gelatinous appendage, similar to the apical appendage, attached obliquely at the lower end and directed toward the flattened side of ascospore. Total length of two-celled ascospore, measured from apex to oblique lower end, 28–35 μ. Ascospores accumulating in a mass at the ostiole entangled among perithecial hairs.

Grown on rabbit dung in laboratory at Cambridge, Mass., April, 1897, from dung of Liberian antelope collected in Africa, R. Thaxter FH 1556, TRTC 31640, **Type.**

Peritheciis ovatis vel piriformibus, nigris, superficialibus, numerosis, 200–230 × 120–150 μ, supra in collum brevum crassum pilosum attenuatis. Parietibus atro-brunneis, semitranslucidis, membranaceis. Cellulis parietis parvis, irregularibus, 6–12 μ crassis, obscuris. Pilis peritheciorum 50–100 × 3–4 μ, rectis vel leniter flexuosis, brunneis, ad apicem obtusis in parte superiore dispersis. Ascis octosporis, clavatis, 100–150 × 16–19 μ, superne late rotundatis, ad apicem orbibus praeditis, basi in stipitem brevem attenuatis. Paraphysibus, filiformibus, septatis. Ascosporis biseriatis, initio hyalinis, ovato-ellipticis, non septatis, dein inaequilateralibus inferne curvatis et

obliqualiter uniseptatis, 28–35 × 12–14 μ. Cellula superiore in maturitate atro-brunnea opacaque, 19–23 × 12–14 μ, foramine germinali singulari, orbiculato, apicali, 2 μ crasso praedito et appendice gelatinosa, curvata, subapicali, hyalina, evanescenti, 10 μ longa, 2 μ crassa ornata. Cellula inferiore hyalina, obliqua, ad septum 10–12 μ crassa, appendice gelatinosa, curvata, obliqua, subterminali, hyalina, evanescenti, 10 μ longa, 2 μ crassa ornata.

*3. **Triangularia bambusae*** (van Beyma) Boedijn. Figs. 19–22

When grown on modified Leonian's agar the colonies spread rapidly with a sparse aerial growth and indistinct margin. The medium was slightly discolored by a pinkish tint. On agar with added oatmeal flakes a more luxuriant growth was produced with a fairly dense layer of aerial white mycelium. The medium was changed to a distinct pink color. Perithecia appeared rapidly on both media but were slightly less abundant on the agar with oatmeal. Ascospores were forcibly discharged to the opposite (upper) side of the test tubes. In some of the older cultures there was a tendency for the ascospores to accumulate in a mass at the ostiole of the perithecium.

Hyphae variable in diameter, branching, anastomosing, with cells usually rather long but shorter and isodiametric in the region of the phialides and ascogonia. Phialides scattered on the mycelium or at ends of hyphae, with a small collarette at apex. Phialospores hyaline, smooth, subglobose, 3–4 μ in diameter, forming a small, globose glomerule at apex of phialide, and capable of germinating to form a hypha.

Perithecia scattered or more or less aggregated in clusters, becoming superficial, brownish-black, 300–500 × 200–350 μ, pyriform, narrowed above into a very short, thick, cylindrical or somewhat tapered neck, covered on the upper part with a few brown, septate, flexuous hairs measuring up to 200 μ long and 3–4 μ thick. Peridium of perithecium dark-brown, membranaceous, areolate with darker and thicker areas. Asci eight-spored, cylindrical with a distinct thickened ring in apex, broadly rounded above, tapering gradually below into a stipe, 140–170 × 12–17 μ. Paraphyses filiform, septate, slightly longer than asci, lying parallel in a fascicle from the base. Ascospores uniseriate, at first elliptical or fusiform and one-celled, later flattened on one side and two-celled by the formation of an oblique septum near base, 18–22 × 9–15 μ (total); upper cell becoming yellow, then olivaceous-brown and finally brownish-black, triangular in one side view, 17–22 × 9–12 μ, with circular, subapical germ pore; lower cell hyaline, forming a narrow border along shorter side of upper cell, thin-walled, 2–3 × 10–15 μ, no gelatinous sheath or appendages.

Relationships

The three species included in the genus *Triangularia* all have angular ascospores. In *T. angulospora*, the upper dark cell is symmetrical about a central longitudinal axis and conspicuously narrowed from the angled broadest part toward the pointed end; the hyaline basal cell is short, broad, and curved

to one side. In *T. obliqua*, the upper dark cell is not so distinctly narrowed toward the apex and consequently not so angular. The transverse septum is oblique, however, and the hyaline basal cell is more sharply turned to one side. This species thus occupies a somewhat intermediate position between *T. angulospora* and *T. bambusae* where the septum in the ascospores is more strongly oblique and the basal cell shorter. In addition, the ascospores of the latter species are distinctly flattened so that, viewed in one position, they appear triangular.

These three species of *Triangularia* represent a specialized line that has apparently evolved from a species of *Podospora* (*Sordaria*). The line of separation is probably not very distinct. The characteristic angled nature of the ascospores of *Triangularia* is the most significant diagnostic feature. However, other lines of evolution within *Podospora* exhibit flattened ascospores, though these are quite distinct from *Triangularia* in the nature of the hyaline basal cell. The species in both of these genera have ascospores in which the germ pore is circular, usually subapical, and at the upper end.

The three species of *Triangularia* all have ascospores with a short, broad, and oblique basal cell or primary appendage. In contrast to this, most species of *Podospora* have a more elongated basal cell, but sometimes this may be quite short but narrow or entirely absent. In the former genus the secondary gelatinous appendages have been much reduced. In *T. obliqua*, there is a short one at each end of the ascospore. In *T. angulospora* they are clearly visible only at the upper end. They have not been detected in the third species, *T. bambusae*.

The young ascospores in all three species of *Triangularia* are elliptic and symmetrical indicating a relationship with *Podospora* in which the ascospores are sometimes ellipsoid when young. However, this is the case in only those *Podospora* species that have no primary appendage (hyaline basal cell). In those having a primary appendage, the young ascospores are more elongated at the base so that they are somewhat pestle-shaped.

The species of *Triangularia* are not directly related to *Sordaria fimicola* (Rob.) Ces. and DeNot. and closely related species. In the latter, the ascospores have no appendages and no septa. In addition the germ pore is at the base of the ascospore instead of the apex.

Von Arx and Müller (1) have placed *Triangularia* in the family Xylariaceae, along with *Gelasinospora*, *Bombardia*, *Sordaria*, *Lasiosordaria*, and *Neurospora*. These genera appear to be related to each other but not necessarily at the family level and not to genera typical of the Xylariaceae, a family characterized by the development of the perithecium below a distinctive stroma or clypeus, by ascospores with elongated germ slits, and by asci with a special type of discharge mechanism in the apex. Furthermore, the imperfect stage of all of the above-mentioned genera is typically a *Phialophora*, and a thallospore type of imperfect is known only for *Neurospora* (the *Monilia* stage). The imperfect stages of members of the Xylariaceae, where known, are quite different from either *Phialophora* or *Monilia*.

Triangularia and *Podospora*, as well as the above-mentioned genera, are considered to be more closely related to genera usually placed in the Hypocreaceae (including Nectriaceae). In both of these groups conidia are typically produced on phialides although as a general rule, they are more highly evolved in the Hypocreaceae. The structure and arrangement of the asci as well as the morphology and development of ascocarps are also similar. In taxa of the Xylariaceae there is a greater tendency for the asci to be spread out in a layer lining a large part of the inner peridium of the ascocarp whereas in the other two groups they usually originate from a small area at the base.

Triangularia should be included in the Sordariaceae along with *Sordaria* and *Podospora*. *Bombardia* is more closely related to *Lasiosphaeria* whereas *Gelasinospora* and *Neurospora* may be closer to *Melanospora*.

The three species included in *Triangularia* appear to be a closely related and natural group of species worthy of recognition as a genus separate from *Podospora*. The line of demarcation is, however, not very distinct.

Acknowledgments

The loan of specimens from the Farlow Herbarium (through the courtesy of the late Dr. D. H. Linder) is gratefully acknowledged. We appreciate the assistance of the following: Miss L. K. Weresub in preparing the manuscript, Mr. L. A. Garay with photography, and Prof. G. W. Martin and Dr. A. L. Welden in collecting soil.

References

1. ARX, J. A. VON and MÜLLER, E. Die Gattungen der amerosporen Pyrenomyceten. Beitr. Kryptogamenfl. Schweiz, 11 (1) : 1-434. 1954.
2. BEYMA THOE KINGMA F. H. VAN. Beschreibung einiger neuer Pilzarten aus dem Centraalbureau voor Schimmelcultures II. Zentr. Bakteriol. Parasitenk. 89 (2) : 237-239. 1933.
3. BOEDIJN, K. B. Ueber die neue Gattung *Trigonia* van Beyma thoe Kingma. Ann. Mycol. Notitiam Sci. Mycol. Univ. 32 : 302. 1934.
4. FARROW, W. M. Tropical soil fungi. Mycologia, 46 : 632-646. 1954.
5. MOREAU, C. Les genres *Sordaria* et *Pleurage*. Encyclopedie mycol. 25 : 1-330. 1953.
6. MOREAU, F. et MME MOREAU. Étude du développement du *Triangularia bambusae* (van Beyma) Boedijn. Rev. mycol. 15 : 146-158. 1950.

EXPLANATION OF FIGURES

FIGS. 1–3. *Triangularia angulospora.*

FIG. 1. Ascospores. × 860. FIG. 2. Ascus with ascospores in early stages of development. × 430. FIG. 3. Ascus showing ascospores approaching maturity and with lower cells nearly devoid of contents. × 430.

FIGS. 4–9. *Triangularia obliqua.*

FIG. 4. Two perithecia. × 120. FIG. 5. Outline of cells of perithecial peridium. × 1140. FIG. 6. Two hairs from neck region of perithecium. × 650. FIG. 7. Ascus with mature ascospores. × 700. FIG. 8. Five hyaline, immature ascospores before septum formation. × 1140. FIG. 9. Fourteen mature ascospores. × 1140.

FIGS. 10–15. *Triangularia angulospora.*

FIG. 10. Four ascospores. TRTC 31639. × 900. FIG. 11. One ascospore. TRTC 31638. × 900. FIG. 12. Three perithecia. × 70. FIG. 13. Group of immature perithecia. The one in the upper left shows the ostiole in the center of the perithecium. × 100. FIG. 14. Perithecium and ascospores. × 90. FIG. 15. Crushed fragments of perithecial peridium showing darker patches and indistinct cellular structure. × 900.

FIGS. 16–17. *Triangularia obliqua.*

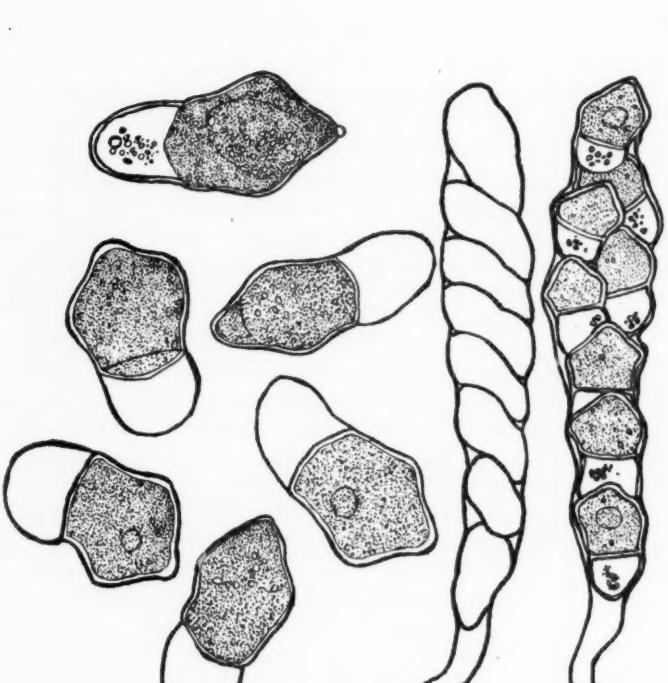
FIG. 16. Crushed perithecium showing hairs around ostiole and ascospores. × 100. FIG. 17. Groups of ascospores. Some are hyaline and immature. × 410.

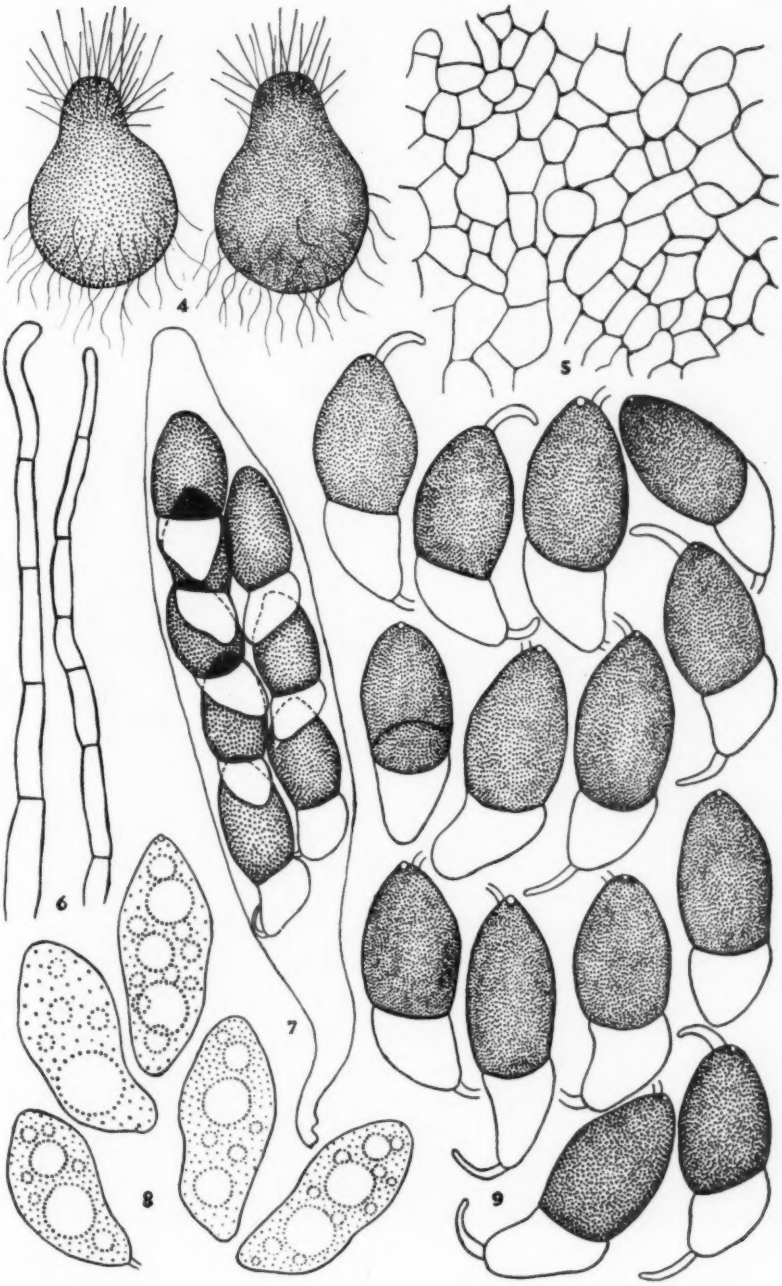
FIG. 18. *Triangularia obliqua.* Ascospores. × 900.

FIGS. 19–22. *Triangularia bambusae.*

FIG. 19. Groups of ascospores. × 410. FIG. 20. Group of asci with ascospores in various stages of development. × 410. FIG. 21. Group of ascospores. × 900. FIG. 22. Two crushed perithecia with asci and ascospores. × 100.

NOTE: FIGS. 1–22 follow.

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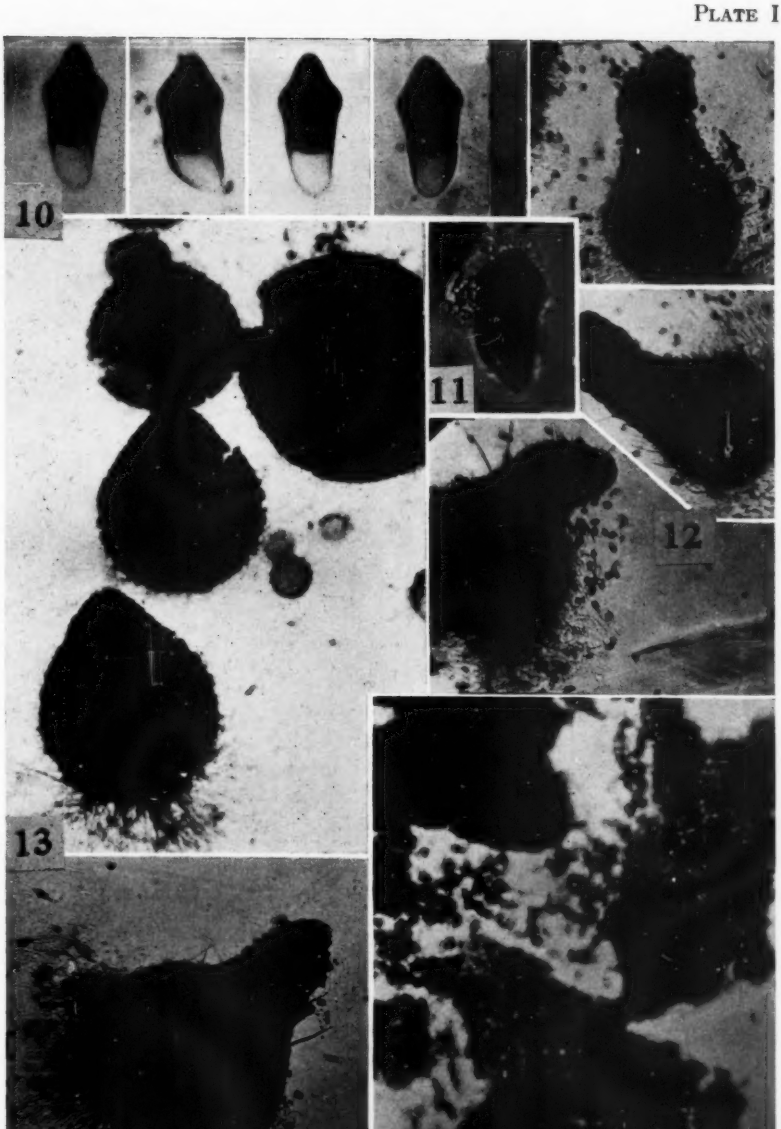

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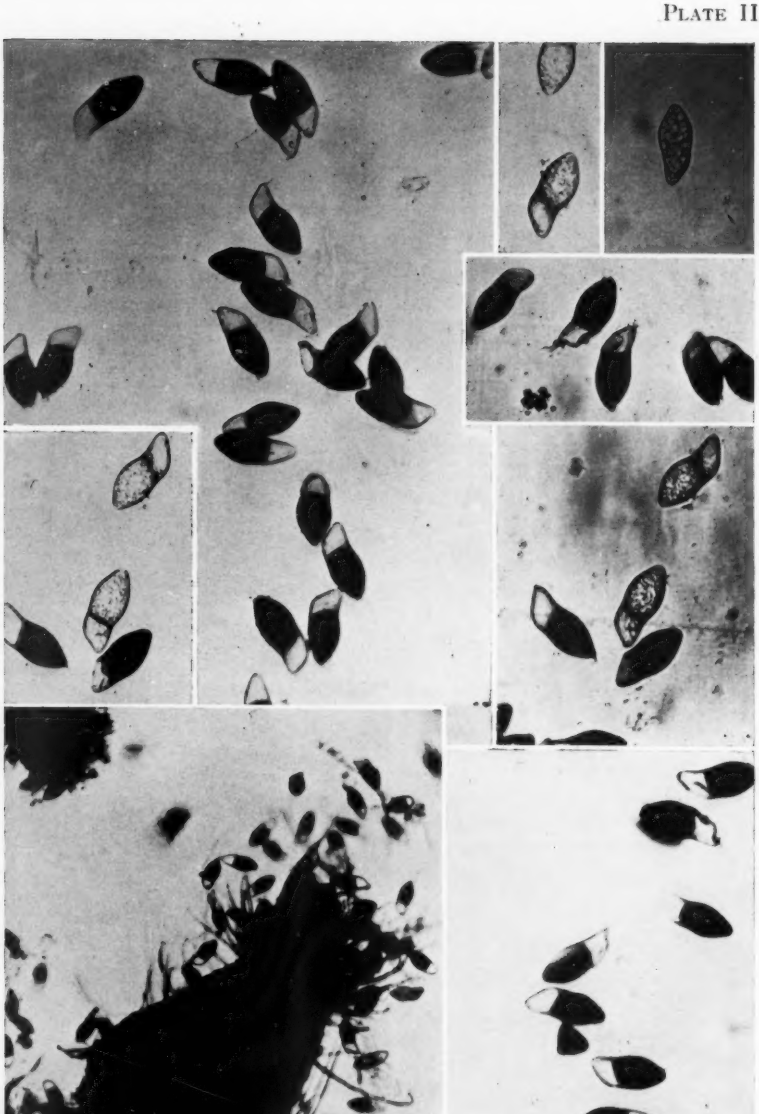

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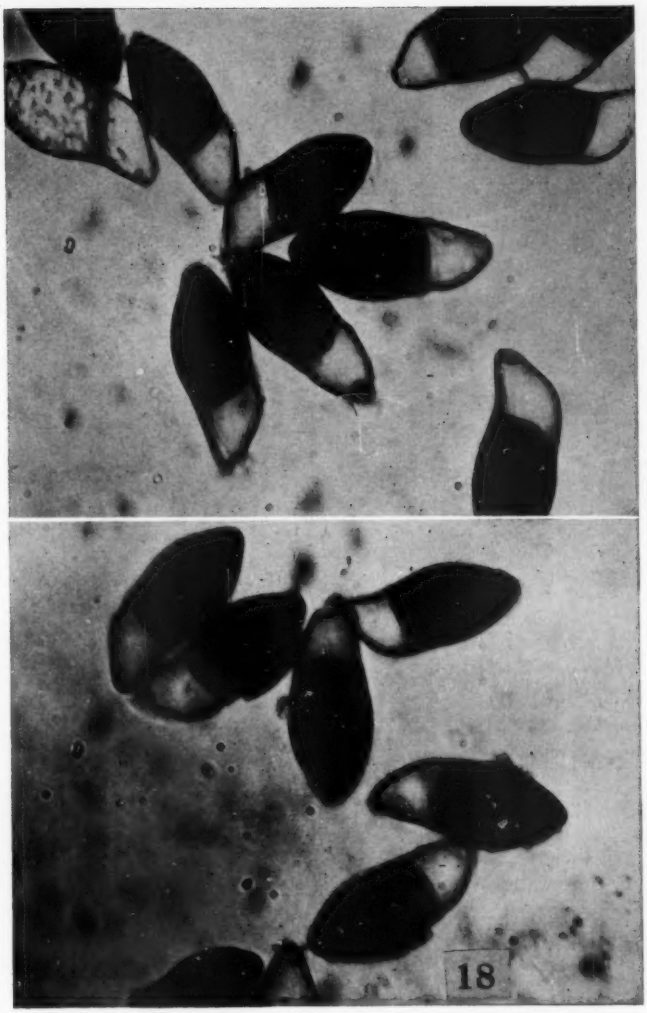
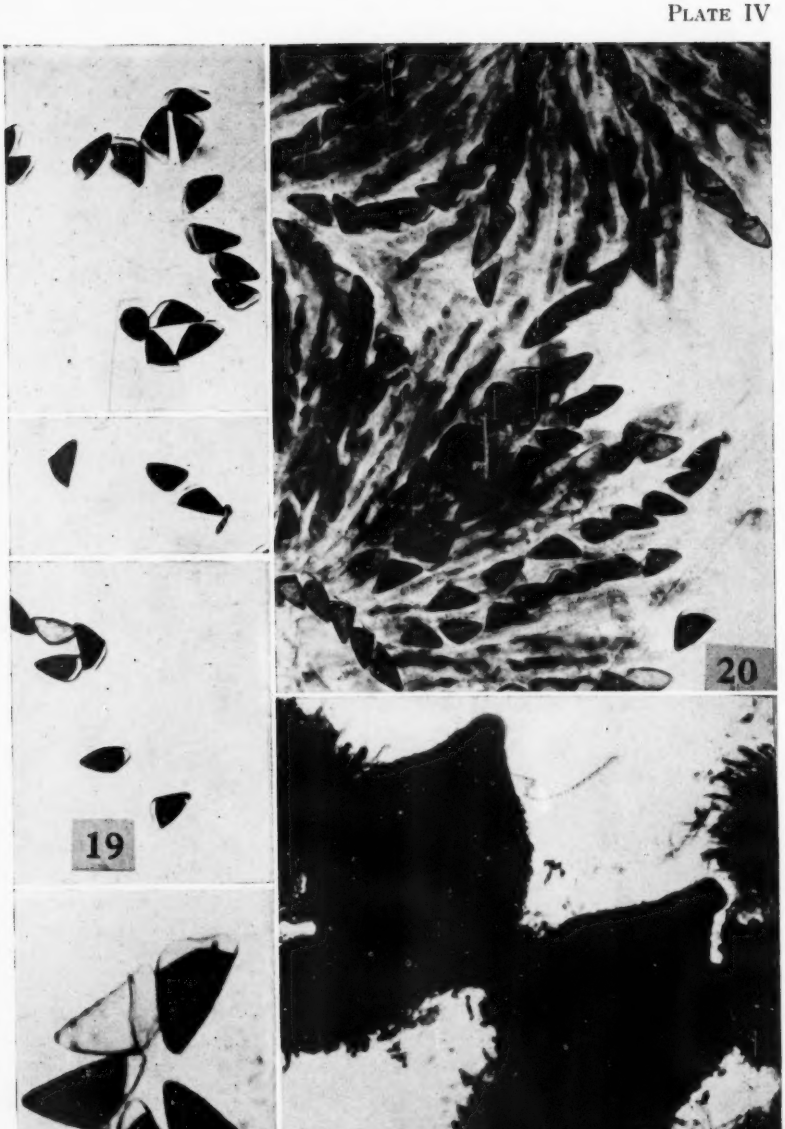
PLATE III





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STUDIES OF COPROPHILOUS ASCOMYCETES

IV. TRIPTEROSPORA, A NEW CLEISTOCARPOUS GENUS IN A NEW FAMILY[1]

By Roy F. Cain[2]

Abstract

A new family Tripterosporaceae is introduced by the description of three species of a new genus, *Tripterospora*, similar in several characteristics to *Podospora* (*Sordaria*) but clearly separable by the production of globose, non-ostiolate ascocarps and of ascospores which lack gelatinous secondary appendages and sheaths. In *T. longicaudata* Cain sp. nov., the type of the genus, and in *T. brevicaudata* Cain sp. nov., early development of the ascocarp is seen to be from a coiled and twisted ascogonium as in many members of the Ascohymeniales, with the ascogonia scattered on the aerial mycelium. Ascospores in all three species are two-celled, the upper cell dark and ellipsoid with a circular germ pore at the apex, the lower cell hyaline. Descriptions and illustrations are given for the known species: *T. longicaudata*, isolated in pure culture from dung and from swiss chard plants in Ontario; *T. brevicaudata*, also observed only in pure culture, obtained from a living branch of yellow birch in Connecticut, and from tomato seed in Ontario; and the third, described by Griffiths as *Pleurage erostrata*, collected on dung in Ontario and Manitoba.

Introduction

A study has been made of three species which resemble the genus *Podospora* in the character of their asci and ascospores. However, these species differ in the evanescence of the ascus wall, the irregular orientation of the asci within the ascocarp peridium, the less extensive development of the paraphyses, and the lack of gelatinous secondary appendages. Furthermore, the ascocarps in all three species are globose, without neck or ostiole. Since there is no genus so characterized, the name *Tripterospora* is proposed.

On account of its inostiolate globose ascocarp, *Tripterospora* should be included in the order Plectascales. Although the writer has pointed out previously (2) that this order is not a natural one, it is, at this time, the only repository for the genus, unless it be included in the ostiolate family Sordariaceae. However, since *Tripterospora* has no close affinity with members of the Aspergillaceae or any other family now known in the Plectascales, it becomes necessary to erect a new family, the Tripterosporaceae.

Descriptions

Tripterosporaceae Cain fam. nov.

Saprophytic; ascocarps globose, superficial, cleistocarpous, developing from ascogonia produced as side branches scattered on mycelium. Asci clavate, in irregular or parallel fascicles, evanescent at maturity. Ascospores dark olivaceous-brown to nearly black, each with a single circular germ pore.

[1]*Manuscript received April 16, 1956.*
Contribution from the Department of Botany, University of Toronto, Toronto, Canada. This study was carried out with the assistance of grants in aid of research from the University of Toronto and the National Research Council of Canada.
[2]*Associate Professor and Curator, Department of Botany, University of Toronto.*

Saprophyticae; ascocarpi globosi, superficiales, sine ostiolo, ab ascogoniis lateralibus dispersis in hyphis orientes. Asci clavati, sive parallele sive in fasciculis irregulariter dispositi, evanescentes. Ascosporae atro-olivaceae, cum foramine germinali singulari orbiculato, praeditae.

Type genus: *Tripterospora* (*tripter*, τριπτήρ, pestle and *spora*, σπορά, seed).

Tripterospora Cain gen. nov.

Saprophytic; ascocarps developing from ascogonia produced as side branches scattered on mycelium, at maturity superficial, globose, without ostiole, covered with flexuous hair-like projections or bare except for surrounding hyphae. Peridium of ascocarp light olivaceous-brown, pseudoparenchymatous, membranaceous to somewhat coriaceous, a few cells in thickness. Asci eight-spored, clavate, stipitate, in irregular fascicles, sometimes with thickened ring in apex, evanescent, leaving spores in mass within ascocarp cavity. Ascospores uniseriate or biseriate, two-celled without gelatinous appendages or sheath; upper cell ellipsoid, smooth, dark olivaceous-brown to nearly black, with circular apical germ pore; lower cell or primary appendage hyaline, without visible contents.

Saprophyticae; ascocarpi superficiales, globosi, pallide olivaceo-brunnei, cum pilis vel hyphis vestiti, ab ascogoniis lateralibus dispersis in hyphis orientes. Peridium ascocarpi pseudoparenchymaticum, membranaceum vel subcoriaceum e cellulis in stratis paucis compositum, non ostiolatum. Asci clavati, octospori, stipitati, evanescentes, ad apicem interdum cum annulo singulo praediti, et in fasciculis irregulariter dispositi. Ascosporae uniseriatae vel biseriatae, biloculares; cellula superior ellipsoidea, levis, atro-olivacea, cum foramine germinali, singulari, orbiculato, apicali praedita; cellula inferior hyalina.

Type species: *Tripterospora longicaudata* Cain.

KEY TO SPECIES

Ascospores with hyaline basal cell broad (more than 4 μ) and short (less than 5 μ)..... 1. *T. brevicaudata*
Ascospores with hyaline basal cell narrow (less than 4 μ) and 6–8 μ long..... 2. *T. erostrata*
Ascospores with hyaline basal cell narrow (less than 4 μ) and 10–17 μ long.. 3. *T. longicaudata*

1. Tripterospora brevicaudata Cain sp. nov. Figs. 1–10

In artificial culture producing white colonies. Hyphae hyaline, 2–8 μ in diameter, branching infrequently, remotely septate. No conidia or spermatia produced. Ascogonia as side branches scattered on mycelium, becoming irregularly twisted and coiled, surrounded by branches from stalk. Ascocarps abundant (Fig. 1), densely aggregated, mostly superficial or partially immersed, some completely immersed in agar matrix, globose, 300–640 μ in diameter, smooth, very light-brown, but appearing black owing to ascospore mass inside, embedded in a loose mat of light flesh-colored hyphae. Peridium of ascocarp very thin, semitransparent, membranaceous to slightly coriaceous, pseudoparenchymatous. Peridial cells (Fig. 2) small, somewhat angular and irregular in shape, very indistinct, in several layers, light-brown, merging on the inside

into the larger, thin-walled, colorless cells surrounding the asci (Fig. 4); outer cells extending into very long, flexuous, fairly thick-walled, remotely septate, rarely branching hairs measuring 2–4 μ in width, light brownish at base, hyaline at apex, with superficial, scattered, irregularly shaped, very faintly brownish crystals. When first crushed out of fully mature ascocarps, spore mass (with asci completely broken down) held together in a single globose ball by a transparent membrane produced by collapsed, hyaline, swollen cells of the innermost peridial layer. Asci (Figs. 5–7) eight-spored, clavate, 140–170 $\times$ 20–28 μ, narrowed somewhat above, with a broadly rounded apex, tapering below to a short stipe with crozier at base, few, forming irregular fascicles, very evanescent, leaving spores in mass in ascocarp cavity. Paraphyses consisting of short chains of swollen cells extending inward from cells of ascocarp peridium. Ascospores (Figs. 5–8) uniseriate or partially biseriate, rapidly becoming biseriate in upper part of ascus when mounted in water, at first hyaline, ellipsoid, with a slightly pointed base, becoming transversely uniseptate; upper cell ellipsoid, 18–26 $\times$ 12–17 μ, finally olivaceous-brown to nearly black, but not quite opaque, with irregular granular contents, rarely with one large refractive globule, with an apical, circular germ pore; basal cell or primary appendage remaining hyaline, 3.5–5.0 μ long and 4–7 μ wide at base, papilliform. Without gelatinous appendages or sheath on ascospores.

The ascospores are usually so oriented in the ascus that the hyaline cells are basal, but sometimes a few spores in the upper end of the ascus are inverted. Another occasional anomaly observed is the failure of an ascospore to form a septum, thereby becoming completely colored but with a papilliform base (Fig. 9).

Monoascospore cultures are self-sterile since they fail to develop mature ascocarps. Ascospores have been seen to germinate after 18 hr. in water, producing a small vesicle outside the germ pore with usually one long and one short branch (Fig. 10). Thereafter, the hyphae, hyaline with a few small oil globules, become quite long before branching.

Collections: Isolated in pure culture at Ottawa, April, 1940, by J. W. Groves, from tomato seed, Eastern Ontario (TRTC 31780). Isolated in pure culture at Ottawa, April, 1940, by J. W. Groves from radish seed (registered), British Columbia (**Type** TRTC 31779). Isolated in pure culture by J. R. Hansbrough, from living branch of yellow birch tree infested with bronze birch borer, Conn., July 22, 1945, (TRTC 31781).

Mycelio ex hyphis hyalinis, raro ramosis, remote septatis, 1–2 μ diam. composito. Neque conidia neque spermatia prolata. Ascocarpis ab ascogoniis lateralibus dispersis in hyphis orientibus. Ascocarpis dense aggregatis, superficialibus, vel raro immersis, globosis, abundantibus 300–640 μ, levibus, pallido-brunneis. Peridio ascocarpi tenuissimo, diaphano, membranaceo vel coriaceo, pseudoparenchymatico. Cellulis peridii in stratis externis pallido-brunneis parvis semiangulatis irregularibusque, valde obscuris; cellulis peridii in stratis internis majoribus, hyalinis, tenuiter tunicatis. Pilis ascocarpi longissimis, flexuosis, crasse tunicatis, remote septatis, raro ramosis, 2–4 μ diam., ad basin pallido-brunneis, ad apicem hyalinis, crystallis vestitis.

Ascosporis in globo singulari conglomeratis. Ascis 8-sporis, clavatis, 140–170 × 20–28 μ, basi in stipitem brevem sensim attenuatis, evanescentibus, in fasciculis irregulariter dispositis. Ascosporis uniseriatis vel partim biseriatis, primum hyalinis, ellipsoideis, basi acumenatis, deinde transverse uniseptatis. Cellula superiore ellipsoidea, in maturitate brunneo- vel atro-olivacea haud opaqua, 18–26 × 12–17 μ, cum foramine germinali singulari, orbiculato praedita. Cellula inferiore hyalina, 3.5–5.0 μ longa, ad basin 4–7 μ lata, papilliformi. Ascosporis sine appendicibus gelatinosis et sine strato mucoso.

2. *Tripterospora erostrata* (Griff.) Cain comb. nov. Figs. 11–16

≡ *Pleurage erostrata* Griff. Mem. Torrey Botan. Club 11 : 71. 1901.

Ascocarps (Fig. 11) scattered, entirely superficial, globose, non-ostiolate, 200–250 μ in diameter, black, opaque, completely covered with very long, flexuous, septate, brown hairs (Fig. 13) measuring about 1 millimeter in length and 4–5 μ in diameter; wall of hair thickened at base, thinner toward apex. Peridium of ascocarp lacking suture marks and special lines of dehiscence, membranaceous, pseudoparenchymatous, composed of dark-brown angular cells in several layers and mostly about 10 μ in diameter (occasionally up to 15 μ) (Fig. 12). Asci eight-spored, clavate, 50–70 × 16–20 μ, forming irregular fascicles, somewhat narrowed in upper part, broadly rounded at apex, with a stipe measuring about 20 μ in length, very evanescent. No paraphyses seen. Ascospores (Figs. 15, 16) mostly biseriate, at first hyaline, one-celled, somewhat pestle-shaped, becoming transversely uniseptate; upper cell finally dark-brown and opaque, ellipsoid, acutely rounded above, with a circular germ pore at apex, broadly rounded to truncate below, 10–12 × 6.5–7.5 μ; basal cell or primary appendage remaining hyaline, cylindrical, straight, 6–8 × 3 μ. Without secondary gelatinous appendages or sheath on ascospores.

Collections: Developed in laboratory, Toronto, Feb. 1940, on rabbit dung collected northwest of Burford, Brant Co., Ont., April 29, 1939, (TRTC 31777). Developed in laboratory, Winnipeg, Man., Jan. 25, 1933, on horse dung collected at Manitoba Agricultural College, G. R. Bisby, 4607 (TRTC 31778).

3. *Tripterospora longicaudata* Cain sp. nov. Figs. 17–33

Colonies on modified Leonian's agar + 0.3% yeast extract, and on "V-8" vegetable juice agar, very similar, at seven days in test tube culture, expanded to a diameter of about 2 in., margin very indefinite, surface irregularly pruinose, with scanty aerial growth, gray in color or with a pinkish cast. Under a lens, aerial mycelium appearing cottony with minute irregular areas. Central half of colony somewhat darkened with ascocarp production and black in reverse. Hyphae hyaline, 1.5–4.0 μ in diameter, frequently anastomosing. No conidia produced. Ascogonia initiated when mycelium is only a few days old, developing rapidly thereafter as side branches scattered on mycelium, becoming irregularly twisted and coiled (Fig. 21), surrounded by branches from stalk which proceed to form peridium of ascocarp, adjoining hyphae not

involved in peridial production, few hyphae extending out from young ascocarp. Ascocarps (Figs. 17–18) densely aggregated, forming a continuous layer over surface of agar, mostly superficial, with a few embedded in agar, globose, 150–250 μ in diameter, covered with a thin, arachnoid weft of mycelium, appearing black and shining by reflected light because of colored ascospores within. Peridium light-brown, pseudoparenchymatous, semi-transparent, coriaceous, about 15–25 μ in thickness. Peridial cells very irregular and interlocking, in three to five layers, the outer (Fig. 19) 4–12 μ in diameter, the inner (Fig. 20) somewhat larger (8–16 μ in diameter). Wall of peridial cells very thin and light yellowish-brown. When first crushed out of fully mature ascocarps, spore mass (with asci completely broken down) held together in a single globose ball by means of a transparent membrane not readily dissolving in water and without cellular structure (Fig. 18) but formed from collapsed hyaline cells of the nutritive layer within the peridium. Asci eight-spored (Figs. 24–27), clavate, 75–85 × 15–18 μ, narrowed somewhat above middle, at maturity showing a distinct thickened ring in broadly rounded apex, narrowed to a short stipe with crozier at base, forming irregular fascicles, not in a single parallel layer or oriented in any single direction, very evanescent. Paraphyses (Figs. 24, 25) consisting of short chains of swollen cells measuring 10–18 × 4–9 μ each containing two or three oil globules, separating freely from asci in crushed mount. Ascospores (Figs. 28–33) biseriate, at first elongate with a swollen upper end, becoming transversely uniseptate; upper cell darkening through olivaceous-brown, with a single refractive oil globule, to nearly black and opaque at maturity, ellipsoid, 13–15 × 8.0–9.5 μ, with an apical, circular germ pore measuring 1.5 μ in diameter; basal cell or primary appendage remaining hyaline, 10–17 × 3 μ, cylindrical, straight or slightly curved. Without gelatinous appendages or sheath on ascospores.

Collections: Isolated in pure culture Jan. 1956, from horse dung collected North of Palgrave, Peel Co., Ontario, Oct. 10, 1955, (**Type** TRTC 31528). Isolated in pure culture by Mary E. Elliott, from plant of swiss chard grown at Ottawa, Ontario, summer 1949 (TRTC 31725).

Mycelio ex hyphis hyalinis, 1.5–4.0 μ diam. composito. Neque conidia neque spermatia prolata. Ascocarpis ab ascogoniis lateralibus, irregularibus, dispersis in hyphis orientibus. Ascocarpis dense aggregatis, superficialibus, vel raro immersis, atris, globosis, abundantibus, 150–250 μ, cum hyphis arachnoideis vestitis. Peridio ascocarpi pallido-brunneo, pseudoparenchymatico, diaphano coriaceo, circa 15–25 μ crasso. Cellulis externis peridii irregularibus, 4–12 μ, pallido-brunneis, tenuiter tunicatis; cellulis internis peridii majoribus, 8–16 μ. Ascosporis in globo singulari conglomeratis. Ascis 8-sporis, clavatis, 75–85 × 15–18 μ, superne late rotundatis, ad apicem cum annulo singulo praeditis, basi in stipitem brevem attenuatis, evanescentibus, in fasciculis irregulariter dispositis. Paraphysibus brevibus cellulis vesiculiformibus, hyalinis, 10–18 × 4–9 μ constitutis. Ascosporis biseriatis, primum pistilliformibus, hyalinis, deinde transverse uniseptatis. Cellula superiore ellipsoidea, in maturitate brunneo- vel atro-olivacea opacoque, 13–15 ×

8.0–9.5 μ, cum foramine germinali, 1.5 μ, singulari, orbiculato praedita. Cellula inferiore hyalina, 10–17 × 3 μ, cylindracea, recta vel subrecta. Ascosporis sine appendicibus gelatinosis et sine strato mucoso.

Relationship

The three species included here in *Tripterospora* appear to be fairly closely related. The superficial ascocarps are much alike although larger in *T. brevicaudata* than in the other two species. The hairs on the ascocarp of *T. erostrata* are longer, broader, and with a thicker wall than those of *T. brevicaudata*, while in *T. longicaudata*, there are no hairs distinct from the hyphae. The ascospores provide the most distinct means of separation. *T. brevicaudata* has the largest ascospores, the dark cell being 18–26 × 12–17 μ; *T. longicaudata* is intermediate with a measurement of 13–15 × 8.0–9.5 μ; and in *T. erostrata*, the dark cell is 10–12 × 6.5–7.5 μ. The respective measurements of the hyaline basal cell of the ascospores for the above three species are as follows: 3.5–5 × 4–7 μ, 10–17 × 3 μ, and 6–8 × 3 μ, the first measurement given indicating the length, the second the width taken at the septum. *T. longicaudata* is distinguished by having the longest basal cell; *T. brevicaudata*, the broadest. The paraphyses are of no particular diagnostic significance, being either absent or short and inconspicuous.

Here, then, we have three species whose inostiolate ascocarps would place them, as mentioned above, in the present-day order Plectascales. But the "cleistothecium" of *Tripterospora* develops from an ascohymenial type of ascogonium, coiled and twisted, and at approaching maturity encloses clavate asci with a thickened apical ring such as are characteristic of *Podospora* (*Sordaria*) (1). The arrangement of the asci within the ascocarps of *Tripterospora* is intermediate between that of *Podospora* and typical members of the Plectascales. The same can be said for the paraphyses: prominent in *Podospora*, reduced to a few short chains of cells in *Tripterospora*, and obsolete in most of the Plectascales. It appears that the cleistothecial habit of this new genus has been of fairly recent origin from an ostiolate member of the Ascohymeniales, quite possibly a taxon very similar to, if not identical with, the present-day species of the genus *Podospora*.

The evolution in ascospore structure, too, seems clear. The *Tripterospora* spore is a *Podospora* type which has lost its gelatinous secondary appendages.

The species of the genus *Zopfiella* Winter (4) may represent a further evolution by the addition of a septum in the dark, ellipsoid cell, with retention of the hyaline appendage as a single cell or its division also into a bicellular structure. Further treatment of this genus will be left to a future publication.

Meanwhile it is important to point out once again that a family like the Tripterosporaceae, which would be incorporated by today's schemes of classification in the Plectascales, is almost certainly in the evolutionary line of certain Ascohymeniales, since so many of the characteristics considered to be indicative of homology are actually examples of parallel evolution between more or less distinct groups.

Acknowledgments

For their contribution of cultures used in this study the writer is deeply indebted to the following: Dr. G. R. Bisby, Miss Mary E. Elliott, Dr. J. W. Groves, and Dr. J. R. Hansbrough. For their assistance in preparing stained sections and revision of the manuscript I am very grateful to Miss Luella K. Weresub and Prof. J. F. Morgan-Jones.

References

1. CAIN, R. F. Studies of coprophilous Sphaeriales in Ontario. Univ. Toronto Studies, Biol. Ser. No. 38 : 1-126. 1934.
2. CAIN, R. F. Studies of coprophilous Ascomycetes. II. *Phaeotrichum*, a new cleistocarpous genus in a new family, and its relationships. Can. J. Botany, 34 : 675-687. 1956.
3. GRIFFITHS, D. The North American Sordariaceae. Mem. Torrey Botan. Club, 11 : 1-134. 1901.
4. WINTER, G. Die Pilze. *In* Rabenhorst's Kryptogamen Flora 1 (2) : 56. 1887.

EXPLANATION OF FIGURES

FIGS. 1–10. *Tripterospora brevicaudata.*

FIG. 1. Ascocarp × 30. FIG. 2. Cells of ascocarp peridium. × 1300. FIG. 3. Irregularly coiled ascogonium with branches beginning to form the ascocarp peridium. × 900. FIG. 4. Nutritive cells from inner part of young ascocarp, before ascus formation. × 800. FIG. 5. Young ascus with hyaline ascospores. × 1300. FIGS. 6, 7. Asci with ascospores nearly mature. × 1300. FIG. 8. Five mature and two immature ascospores. × 1300. FIG. 9. One mature ascospore in which the septum has not been developed. × 1300. FIG. 10. Mature ascospore germinating after 18 hr. in water. × 600.

FIGS. 11–16. *Tripterospora erostrata.*

FIG. 11. Ascocarp. × 60. FIG. 12. Cells of ascocarp peridium. × 900. FIG. 13. Mid, apical, and basal portion of hair from ascocarp peridium. × 900. FIG. 14. Ascus with nearly mature ascospores. × 900. FIG. 15. Mature ascospores. × 900. FIG. 16. Mature ascospores. × 1300.

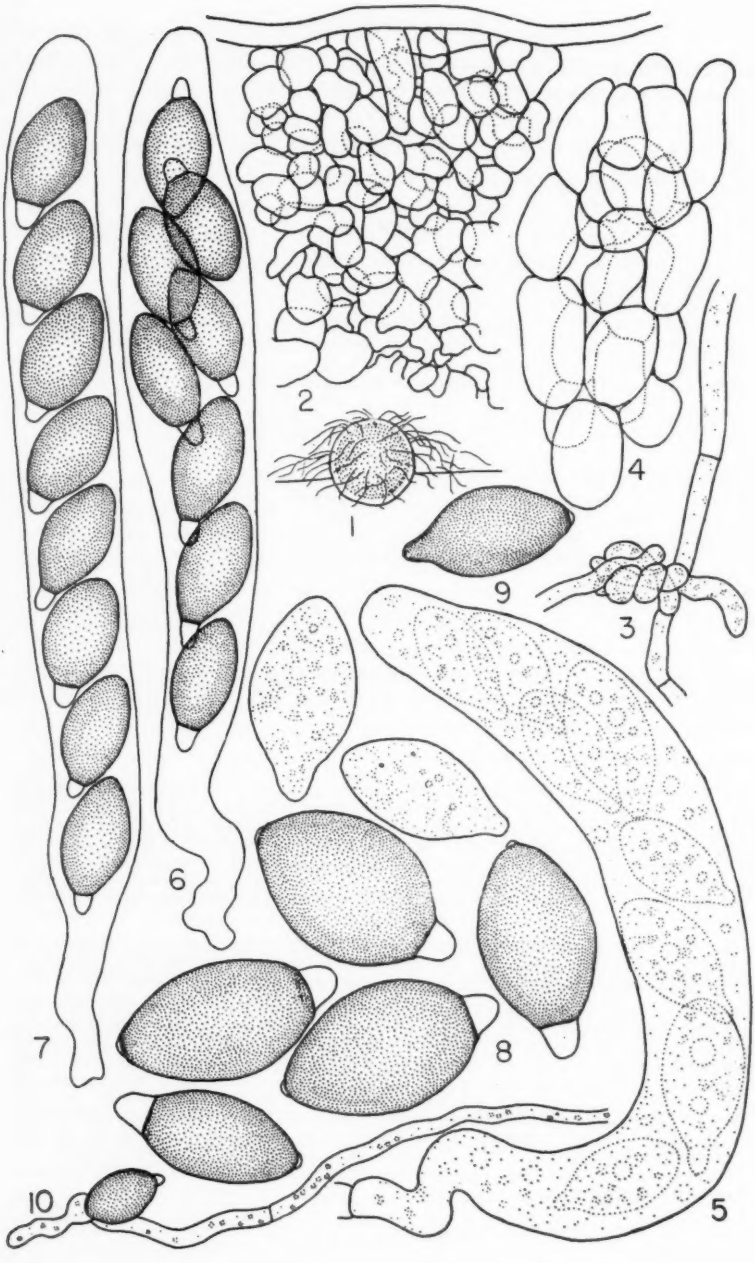
FIGS. 17–33. *Tripterospora longicaudata.*

FIG. 17. Ascocarp. × 100. FIG. 18. Ascocarp crushed open with globose mass of ascospores extruded but held together by membrane. Asci have broken down. × 100. FIG. 19. Outer layer of cells from ascocarp peridium. × 1140. FIG. 20. Inner layer cells from ascocarp peridium. × 1140. FIG. 21. Hyphae with coiled and twisted ascogonia, four of which have branches from stalk beginning to form peridium. × 1140. FIG. 22. Ascogonial cells with croziers and young asci in various stages of development. × 1140. FIG. 23. Crozier on ascogonial cell with four nuclei stained with aceto-orcein. × 1140.

FIG. 24. Paraphyses and asci, one very small and immature, four just after ascospore delimitation. × 1140. FIG. 25. Paraphyses and three asci with immature ascospores. × 1140. FIG. 26. Two asci with ascospores just prior to septum formation. × 1140.

FIG. 27. Asci, one with nearly mature ascospores and one after ascospore discharge, each showing thickened ring in apex. × 1140. FIG. 28. Seven young hyaline ascospores at various stages of development before septum formation. × 1140. FIG. 29. Eleven ascospores not fully mature but after septum formation. Upper cell is light olivaceous-brown with refractive oil globule. × 1140. FIG. 30. Twenty-one ascospores, fully mature, many with basal cell collapsed. Apical germ pore visible in some. × 1140. FIG. 31. Giant ascospore, produced singly in an ascus, upper cell is black and opaque, lower hyaline. Note the short, broad, triangular basal cell. × 1140. FIG. 32. Mature ascospore in longitudinal optical section showing thickness of wall and apical germ pore. × 1140. FIG. 33. Two mature ascospores, optical section, end view showing thickness of wall and germ pore in one. × 1140.

NOTE: FIGS. 1–33 follow.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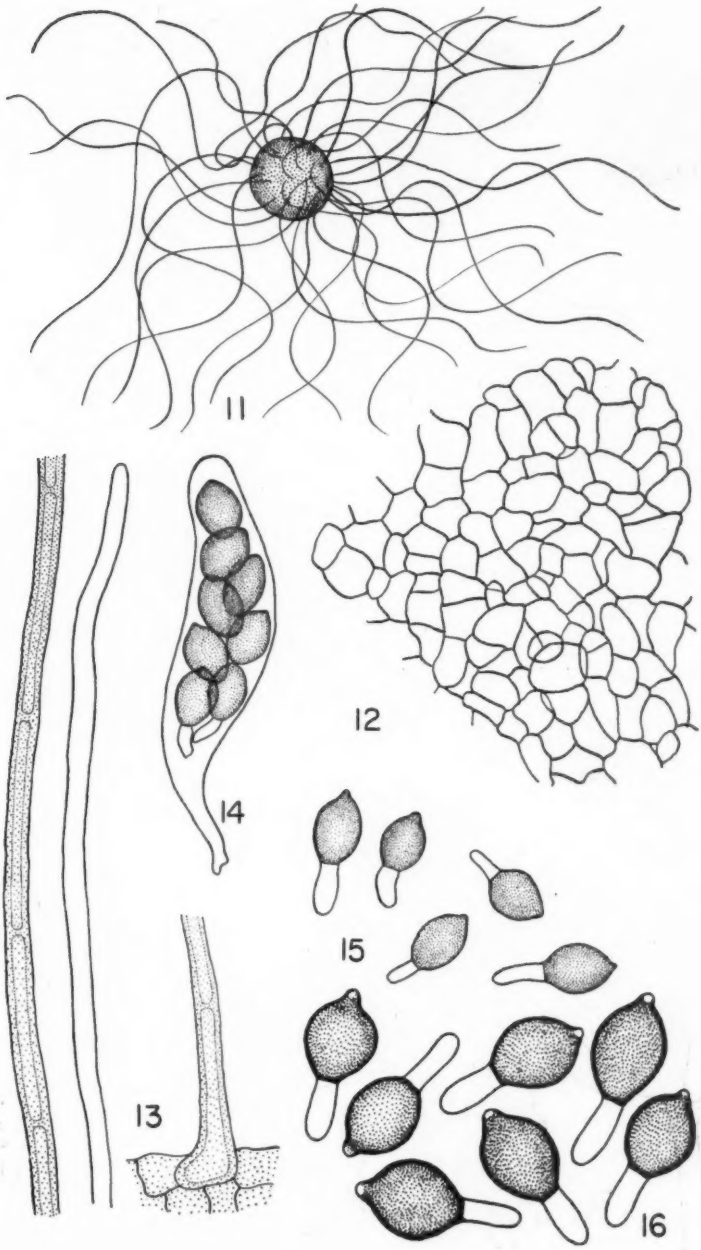
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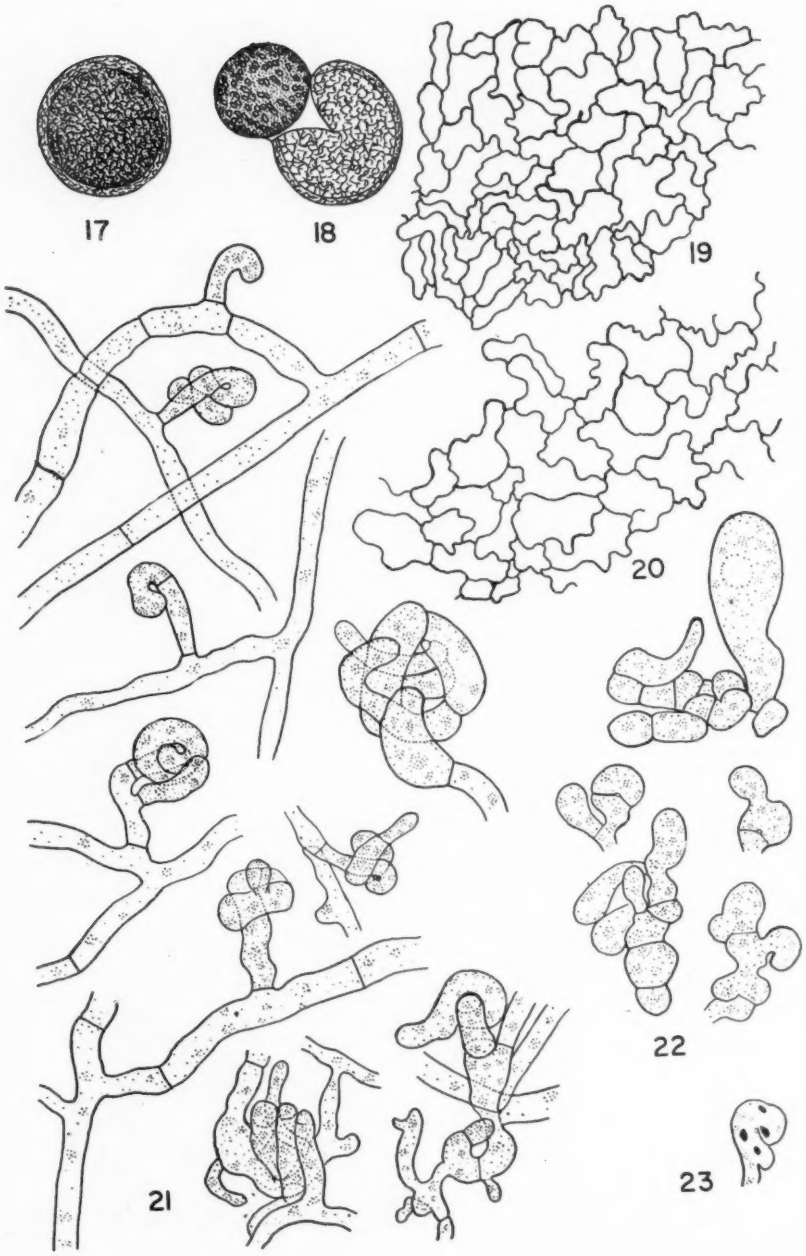
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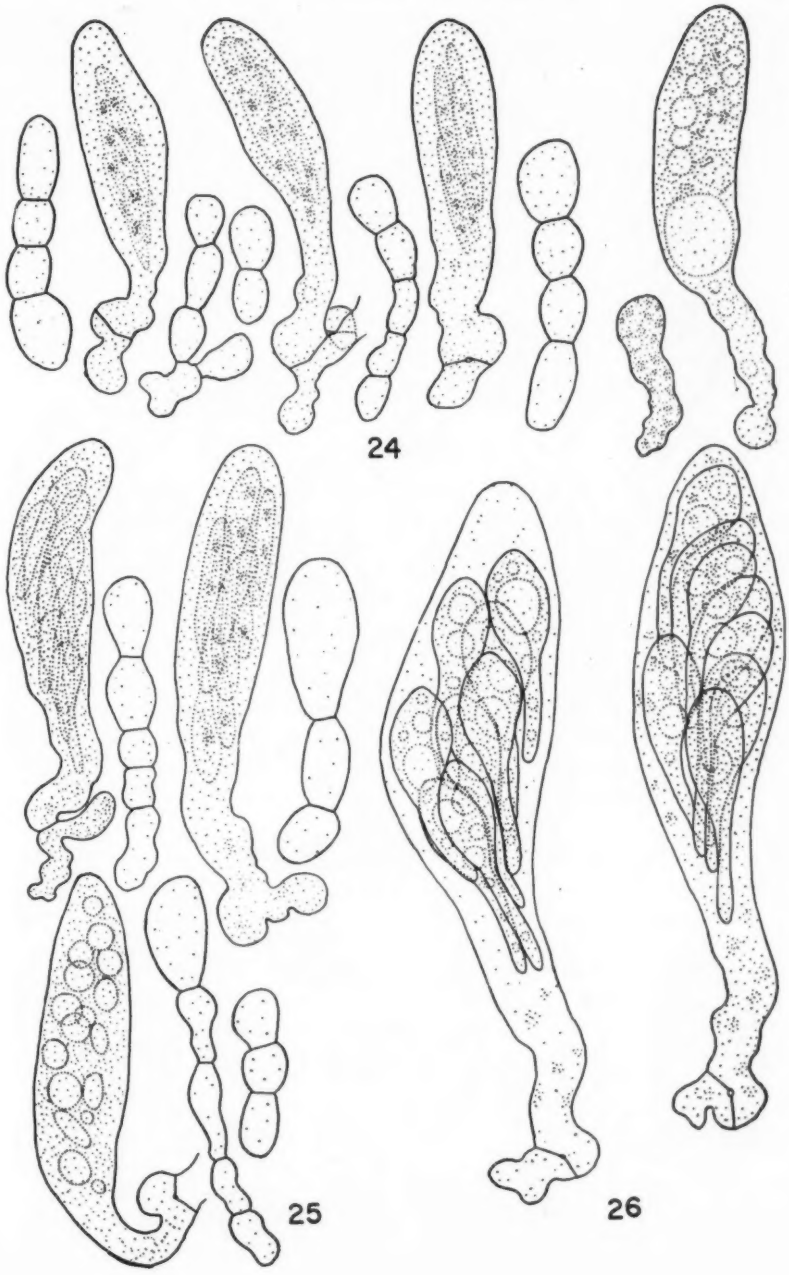

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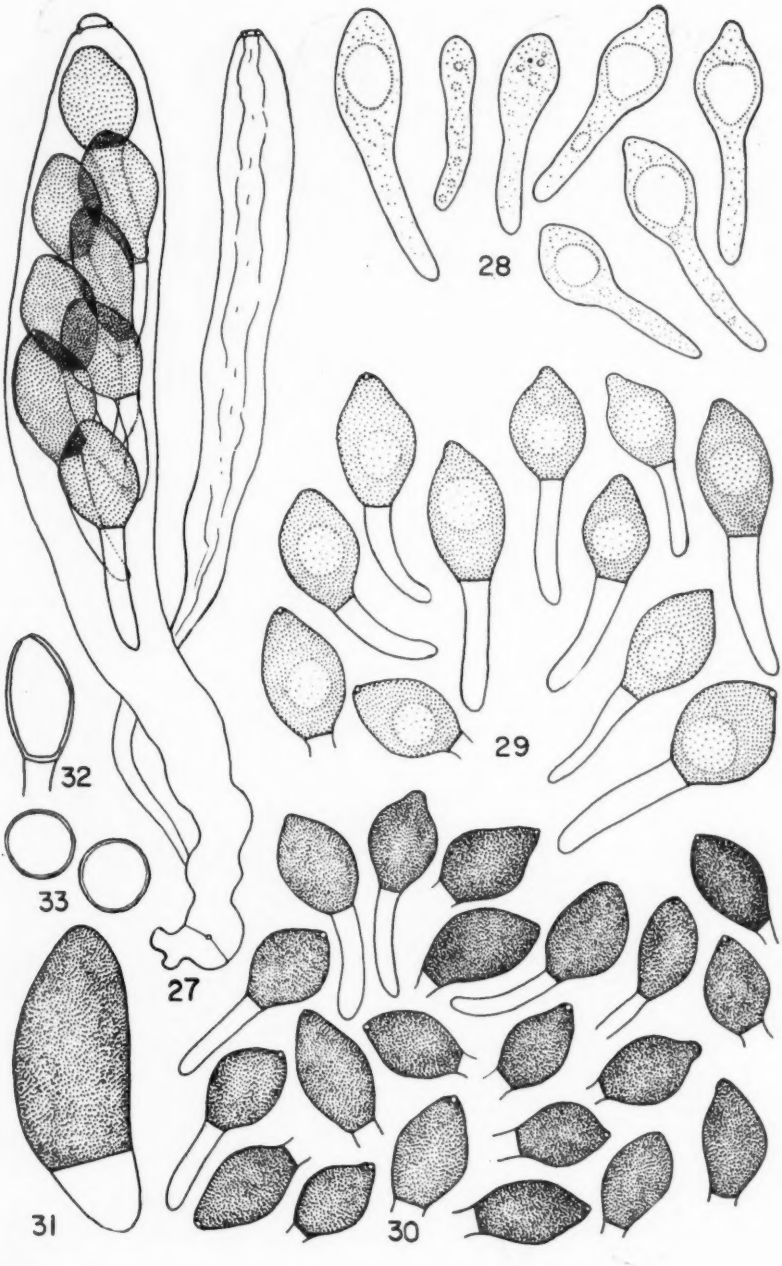
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SELECTION TECHNIQUES IN SCREENING FOR COUMARIN-DEFICIENT SWEET CLOVER PLANTS[1]

By B. P. Goplen,[2] J. E. R. Greenshields,[3] and W. J. White[4]

Abstract

The loss of coumarin from sweet clover leaf samples amounted to 70 to 75% when the samples were air-dried for 10 days and 21 to 51% when oven-dried at 175° F. for one hour. Fresh leaf material placed in 2.5 *N* NaOH and stored in darkness at room temperature showed no loss of coumarin over a six month period but when stored in light at room temperature losses were evident in 10 days and very heavy after six months' storage. Coumarin content was maximum in flower buds and fresh leaves from the tip of branches. Root and stem tissue and the more mature leaves from the central and lower portion of the plants were very low in coumarin. A rapid qualitative procedure is described which involves collection of fresh young leaves and storage of them in 2.5 *N* NaOH in the dark until they are examined in ultraviolet light. Plants classified as coumarin-deficient by this procedure were found to be void of coumarin on photofluorometric analysis.

Introduction

At the Forage Crops Laboratory the breeding of a coumarin-deficient variety of sweet clover has received high priority in the improvement program with that crop. To a considerable degree the effectiveness of selection for the coumarin-deficient character is dependent upon employing reliable sampling and testing techniques. Because it is generally necessary to work with large populations the procedure should be as simple and rapid as is consistent with reliability. Although there have been developed methods of sampling and testing for coumarin in sweet clover plant tissues which are relatively rapid and simple, experience indicated that variations were occurring which were apparently traceable to technique. Techniques of sampling plant material and of storage of samples were therefore studied in an attempt to increase the reliability of methods of screening for coumarin-deficient plants.

Literature Review

Methods of Testing for Coumarin

A number of different methods have been used to analyze plant material for coumarin content. Some of these methods (4, 5, 6, 8) are too cumbersome to be utilized by plant breeders on large populations of plants. The colorimetric procedure (3), developed originally by Clayton (2), is rapid but it measures only the free coumarin and fails to detect bound coumarin (13). Slatensek and Washburn (13) reported that with hydrolysis of the leaf material prior

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Contribution from the Forage Crops Division, Experimental Farms Service, Canada Department of Agriculture, Saskatoon, Sask. This paper was adapted from a portion of a thesis submitted by the senior author to the College of Graduate Studies, University of Saskatchewan, Saskatoon, in partial fulfillment of the requirements for the degree of Master of Science.
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to alcohol extraction of the coumarin, the colorimetric method measured total coumarin content. In recent years the fluorometric procedure, originally described by Ufer (16), has been used quite extensively. Slatensek and Washburn (13) described modifications of Ufer's method and White *et al.* (18) outlined a rapid method which was a variation of Slatensek and Washburn's procedure. With the fluorometric procedure coumarin content can be measured quantitatively on a photofluorometer (12, 13) or semiquantitatively (13, 16, 18) by visual assessment of the fluorescence when samples are placed in ultraviolet light.

Methods of Sampling

Among workers considerable variation has existed in the sampling procedures used and in the method of handling samples between collection and analysis. The size of sample used has varied from 20 gm. of dry leaves (8) to four disks cut from fresh leaves, the disks being about 6 to 7 mm. in diameter (11). In most of the more recent work the sample size has been relatively small (9, 12, 13, 18).

Most investigators have used leaf material (8, 9, 10, 11, 13, 16, 17) but Duncan and Dustman (5) used sections of stems. It has been generally observed that the coumarin content of leaves is higher than that of stems and often the difference is large (1, 7, 12, 14). However, Kanewskaja and Fedorowa (8) found no consistent difference in the coumarin content of these two tissues. Leaves from different regions on the plant have been shown to differ widely in coumarin content (1, 7, 12, 17). Young fresh leaves have a considerably higher content than old leaves. Flower buds and flowers have a high content (17).

A number of workers (4, 8, 9, 13, 18) have followed the procedure of air- or oven-drying the plant material prior to testing it. Other investigators have used fresh material (10, 11, 16, 17). Stevenson and Clayton (14) showed that considerable coumarin was lost in oven- or air-drying.

Coumarin content has been shown to vary with stage of growth (7, 12, 14, 15, 17). Generally the highest content has been observed to occur at approximately the early flowering stage.

Materials and Methods

Plants of white blossom sweet clover (*Melilotus alba*) were used in this study. Most of the plants utilized were of the Arctic variety and contained free coumarin. A few plants of the Pioneer variety were used and these contained bound coumarin only. Samples were collected at the early flowering stage from plants growing both in the field and in the greenhouse.

Only the fluorometric method of analysis for coumarin was used as it provides a quantitative measure irrespective of whether the coumarin is in the free or the bound state. The procedure followed, modified somewhat from that described by Slatensek and Washburn (13) and by Slatensek (12), was as follows. One hundred milligrams of fresh material was placed in a test

tube containing 15 ml. of 2.5 *N* sodium hydroxide and loosely corked. The test tubes and contents were heated for two and one-quarter hours at 97° C. after which they were cooled to about 20° C. using tap water as the cooling agent. Then, using an automatic pipette, 1 ml. of the solution was taken and placed into a volumetric flask containing 99 ml. of distilled water and thorough mixing was accomplished by tipping the flask. The solution was placed into a cuvette and irradiated before an ultraviolet lamp for 10 min. and immediately read in a Coleman photofluorometer, model 12B, equipped with B-1 and PC-1 filters. Prior to taking the reading of an unknown sample, the instrument was checked for sensitivity using a 1.00% coumarin standard. The meter values were converted to percentage coumarin by reference to a standard curve.

The standard curve was prepared from the photofluorometer readings obtained on known coumarin standards. Preliminary to preparing these standards, leaves of alfalfa and of coumarin-deficient sweet clover were compared as sources of chlorophyll and no difference could be detected between them. In preparing standards, varying weights of coumarin-deficient sweet clover leaves and of coumarin were used to make up a total weight of 100 mgm. in each case. For example, the 0.50 standard was prepared by taking 99.5 mgm. of leaves plus 1 ml. of a standard coumarin solution containing 0.5 mgm. of coumarin per ml. Standards ranging from 0.10 to

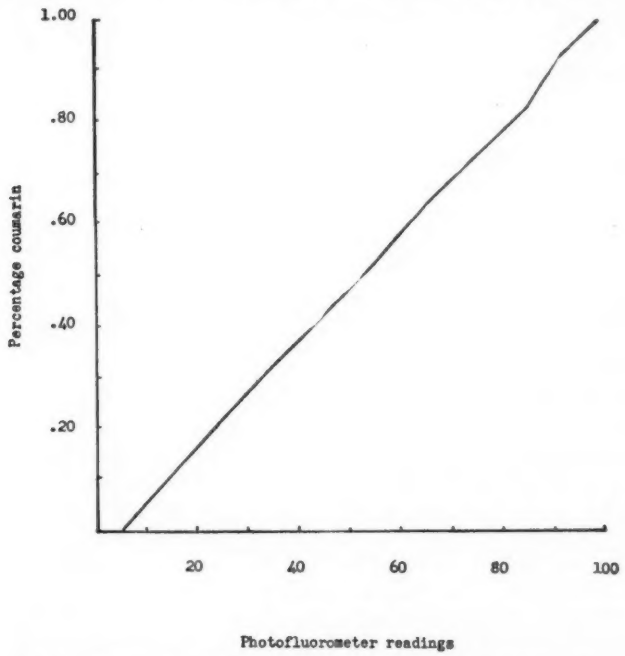

FIG. 1. Standard curve for determination of percentage of coumarin.

1.00% with intervals of 0.10% were prepared in this manner. Blanks containing only diluted solvent also were used to record inescapable intrinsic fluorescence values.

The standard curve is illustrated in Fig. 1. The zero standard as well as the blank (diluted solvent only) gave a reading of about five units on the photofluorometer. From this base point the standard curve shows a straight line relationship between coumarin content and photofluorometer reading. The small deviations from the straight line are likely due to slight inaccuracies in dilution procedure and/or fluctuations in voltage supply to the photofluorometer.

Experimental Results and Discussion

It has been shown by White and Horner (17) and by Slatensek (12) that coumarin content varies with stage of development of plants. Thus to secure reliable data on interplant differences in coumarin content it is important to sample the population at the same stage of development. Where the plant population is large the speed at which sampling should be done may considerably exceed the rapidity with which testing can be accomplished by the rather lengthy photofluorometric procedure. To circumvent this problem a possible procedure is to collect the samples as rapidly as necessary and store them for future testing. A study was therefore undertaken to determine whether storage affected the coumarin content and if so to try to develop suitable storage conditions.

The Effect of Drying on Coumarin Content of Leaf Samples

Drying is a method of preservation of samples which, as indicated previously, has been used rather frequently by various workers. It has been reported that some loss of coumarin occurs on drying (7, 14), and this has been indicated in some unpublished work at the Forage Crops Laboratory. In the selection of coumarin-deficient plants losses on drying may not seriously hamper the breeding program. But in a critical study of coumarin content such losses may introduce serious errors particularly if the percentage loss is not uniform. Therefore a study was undertaken to ascertain the extent of loss of coumarin on drying sweet clover leaves.

Six plants possessing coumarin were chosen for use in this study. From each plant three 100 mgm. samples were made up by separating the three leaflets of each leaf and using one leaflet for each of the three samples. This procedure provided the maximum assurance that the samples were identical in coumarin content at the outset. One set of samples was immediately analyzed for coumarin content. The second set was dried in an oven at 175° F. for one hour, while the third set was air-dried at room temperature (20° C.) for 10 days. The data on coumarin content is given in Table I.

Both drying treatments resulted in loss of coumarin, but air-drying caused a much larger loss than oven-drying. However, on oven-drying the percentage loss varied widely and such variations would be a disturbing factor in critical

TABLE I

COUMARIN PERCENTAGE (GREEN WEIGHT BASIS) OF FRESH, OVEN-DRIED, AND AIR-DRIED SAMPLES AND PERCENTAGE LOSS ON DRYING LEAVES FROM SIX PLANTS

Plant No.	Variety*	Fresh material	Oven-dried		Air-dried	
		Coumarin content, %	Coumarin content, %	% loss	Coumarin content, %	% loss
1	Arctic	0.36	0.22	39.9	0.10	72.2
2	Arctic	0.47	0.23	51.1	0.14	70.2
3	Pioneer	0.50	0.32	36.0	0.13	74.0
4	Pioneer	0.66	0.41	37.9	0.16	75.8
5	Pioneer	0.50	0.36	28.0	0.15	70.0
6	Pioneer	0.50	0.44	21.4	0.15	73.2

**Plants of the Arctic variety contain free coumarin while plants of the Pioneer variety contain bound coumarin.*

analysis. While, on air-drying, the loss of coumarin was relatively uniform, the loss was large and the interplant range was narrowed so drastically that there would be doubt concerning the significance of differences between plants. The loss of coumarin was not influenced by the type of coumarin (free or bound) which the plant material contained. From these results it is obvious that neither of the two types of drying could be used satisfactorily where accurate measurement of coumarin content was required.

Effect of Storage on Coumarin Content of Leaf Samples in Sodium Hydroxide Solution

Since drying of leaves resulted in an appreciable loss of coumarin, an alternative method of storage was sought. The procedure investigated was that of placing the leaves in sodium hydroxide solution in test tubes immediately upon collection. With samples stored in this way the effect of light, temperature, and oxidation upon coumarin content was studied.

Ten duplicate 100 mgm. samples of fresh green leaves were collected. Each 100 mgm. sample was placed in a test tube containing 15 ml. of 2.5 *N* sodium hydroxide. The coumarin content of a portion of each sample was determined immediately with the photofluorometer. One set of samples was stored in light at room temperature (20° C.), and the duplicate set was stored in the dark in a refrigerator at about 2° C. Portions of each sample were analyzed at 10 day intervals thereafter. This analysis revealed that samples stored in the light at room temperature lost some degree of fluorescence. However, the fluorescence of the samples stored in the refrigerator was not altered by storage up to two months. It was evident that either light and/or room temperature caused a partial destruction of the fluorescent properties of stored samples.

TABLE II

EFFECT OF LIGHT AND DARKNESS DURING STORAGE ON PHOTOFLUOROMETRIC READINGS OF SAMPLES STORED IN NaOH AT ROOM TEMPERATURE

Plant or standard sample tested	Stored in light			Stored in darkness		
	Initial reading	Reading after storage for		Initial reading	Reading after storage for	
		10 days	6 mos.		10 days	6 mos.
Arctic	38	32	24	32	33	33
Arctic	49	45	10	45	47	46
Pioneer	67	60	26	55	57	55
Pioneer	52	45	6	48	48	48
Pioneer	57	50	9	45	46	46
Pioneer	52	43	16	51	51	51
1.00% + s.c. leaves	100	91	25	100	99	96
1.00% + alf. leaves	102	91	9	97	97	93
1.00%—solvent only	101	102	5	102	103	102
1.00%— " "	105	105	10	98	100	100

A second trial was then set up to study the effect of light alone. The procedure followed was identical to that given above except that one set of samples was stored in light at room temperature and the duplicate in the dark at room temperature. The results of the analysis are given in Table II.

The data in Table II show that the samples exposed to light gave lower photofluorometric reading even after 10 days' storage and a very markedly reduced reading after six months' storage. In striking contrast, the photofluorometric readings of samples held in darkness were unaltered by storage up to six months. This indicated that in the presence of light a photochemical reaction occurred which resulted in decrease of the fluorescence property of sodium coumarate.

Another set of samples was subjected to intense oxidation in the dark at room temperature. This treatment did not change the fluorescence properties. Thus the decrease of fluorescence with storage in light could not be attributed to oxidation.

The results discussed above establish that samples can be stored in 2.5 *N* sodium hydroxide at room temperature in darkness for periods up to six months without loss of their fluorescent properties.

Distribution of Coumarin in Various Plant Parts

A number of workers (1, 7, 12, 14, 17) have shown that coumarin content varies greatly in different plant tissues. With the existence of variation of this nature adequate sampling presents a problem. As a basis on which to evolve a suitable sampling procedure a study was undertaken of the distribution of coumarin in various parts of the plant.

Eight plants growing in the greenhouse and eight plants growing in the field were used in this study. For each tissue tested, samples of 100 mgm., were used and coumarin content was determined with the photofluorometer. The results are given in Tables III and IV.

The data presented in Tables III and IV show that on each plant the coumarin content varied from very small amounts to quite high concentrations, depending upon the tissue sampled and the age of the tissue. Flower buds and fresh leaves from the tip of branches invariably gave the highest values. The lower portion of the stem, the roots, and the more mature leaves from the middle and particularly the lower parts of the plants were consistently very low in coumarin content. The upper portion of the stem gave intermediate values. Root and stem tissue would not generally be used in sampling since leaves are easier to collect. It is obvious, however, that the leaves must be collected from the tip of branches to obtain a measure of maximum coumarin content and to provide accuracy. On the plants from the greenhouse the

TABLE III

COUMARIN CONTENT (GREEN WEIGHT BASIS) OF VARIOUS PARTS OF EIGHT SWEET CLOVER PLANTS SAMPLED AT THE EARLY FLOWERING STAGE (GREENHOUSE-GROWN)

Part of plant sampled	Per cent coumarin in plant number							
	1	2	3	4	5	6	7	8
Young leaves from tip main stem	0.73	0.50	0.74	0.57	0.66	0.83	0.73	0.61
" " " tip branch 1	—	—	—	—	0.54	0.81	0.55	0.53
" " " " " 2	—	—	—	—	0.43	0.76	1.10	0.38
" " " " " 3	—	—	—	—	0.50	0.52	0.72	0.32
Leaves from mid-section branch 1	0.09	0.11	0.20	0.08	—	—	—	—
" " " " 2	0.20	0.02	0.24	0.14	—	—	—	—
" " " " 3	0.11	0.09	0.29	0.11	—	—	—	—
" " " plant	0.07	0.09	0.15	0.12	0.15	0.06	0.08	0.06
" " lower part	0.05	0.00	0.05	0.03	0.14	0.05	0.05	0.03
Stem—upper portion	0.42	0.39	0.52	0.62	0.30	0.46	0.25	0.34
Stem—lower portion	0.07	0.05	0.10	0.06	0.06	0.06	0.04	0.03
Flower buds	0.77	0.49	0.73	0.96	0.55	0.77	0.63	0.62
Root	0.12	0.04	0.07	0.05	0.10	0.09	0.11	0.08

TABLE IV

COUMARIN CONTENT (GREEN WEIGHT BASIS) OF VARIOUS PARTS OF EIGHT SWEET CLOVER PLANTS IN THEIR FIRST YEAR OF GROWTH IN THE FIELD

Part of plant sampled	Per cent coumarin in plant number							
	1	2	3	4	5	6	7	8
Young leaves from tip main stem	0.76	0.51	0.86	0.76	0.76	0.66	0.15	0.64
" " " branch 1	0.77	0.56	0.85	0.78	0.77	0.62	0.11	0.53
" " " " " 2	0.80	0.51	0.87	0.81	0.39	0.61	0.15	0.58
" " " " " 3	0.81	0.56	0.88	0.77	0.71	0.70	0.14	0.57
Leaves from mid-section plant	0.20	0.15	0.06	0.07	0.06	0.06	0.01	0.04
" " lower part	0.24	0.16	0.03	0.03	0.05	0.02	0.01	0.06
Stem—upper portion	0.33	0.34	0.42	0.35	0.32	0.41	0.27	0.19
Stem—lower portion	0.09	0.02	0.04	0.04	0.03	0.03	0.02	0.04
Flower buds—'bolted'	0.44	0.43	0.49	0.50	0.53	0.51	0.30	0.50
Root	0.08	0.15	0.05	0.21	0.08	0.17	0.06	0.07

flower buds gave values essentially similar to those obtained from fresh leaves from the tips of branches. The flower buds on the plants growing in the field were obtained in the early fall of the first year of growth. They were abnormal in appearance, which may account for the fact that the coumarin content of this tissue was lower than in fresh leaves from the same plants.

On the field grown plants, fresh leaves from the tip of each of four branches were sampled. As shown in Table IV, with one exception, the values obtained on these four leaf samples from each plant corresponded very closely. This shows that when the sample consists of fresh leaves from the tips of branches good repeatability of results is obtained. With plants growing in the greenhouse, the variation was considerably greater between samples of the leaves from the tip of different branches. The coumarin content of leaves from the tip of the main branch was usually higher than that found on lateral branches. This variation on plants in the greenhouse is attributed to the fact that, under the conditions provided, the plants produced one well-developed main branch and several poorly-developed lateral branches. In contrast, the field grown plants generally possessed lateral branches apparently equal in growth to the main stem.

On the basis of these results, it is obviously advisable to sample only the actively-growing leaves from the tip of branches. Such leaves can be taken from any branches displaying growth and vigor about equivalent to that of the main stem. By following this sampling procedure a reliable measure of maximum coumarin content is obtained. Employment of this procedure is particularly important in screening a population for plants which are coumarin-deficient.

A Modified Rapid Fluorometric Method of Coumarin Analysis

The rapid fluorometric procedures described by Slatensek and Washburn (13) and by White *et al.* (18) involve drying of the leaf samples prior to testing. On the basis of results given in Table I it is obvious that losses of coumarin during drying of leaf samples could result in low-coumarin plants being classified as coumarin-deficient. Since, as shown in Table II, leaf samples could be stored for prolonged periods in a sodium hydroxide solution a procedure was developed embodying this method of storage. The procedure is adapted for testing large populations of single plants.

The procedure adopted is to collect fresh young leaves from the tip of the branches at a stage when the plants are growing actively. From each plant two or three such leaves are selected and immediately placed in a $\frac{3}{4}$ in. $\times$ 6 in. test tube containing about 10 ml. of 2.5 *N* sodium hydroxide. A rack carrying 250 test tubes can be conveniently handled. The test tubes are stored at room temperature in the dark until they can be tested. They are then heated for two and one-quarter hours at 97° C., cooled to room temperature, and examined in ultraviolet light. While not quantitative, this procedure will screen coumarin-deficient plants from those containing even small quantities of coumarin. Plants containing as low as 0.07% coumarin

have been found to display a distinct bright green fluorescence which is the characteristic fluorescence of coumarin.

Using this procedure over 2000 plants have been screened for coumarin content. Plants found to be coumarin-deficient in the initial examination were retested by the same procedure and later rechecked by photofluorometric analysis. The retesting confirmed the initial classification of the plants as coumarin-deficient. Thus it appears that the procedure is reliable.

Acknowledgments

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References

1. Brink, V. C. The content, distribution, and some metabolic aspects of coumarin in sweet clover (*Melilotus alba* Des.). Summaries of Doctoral Dissertations, Univ. of Wisconsin, 6 : 41-42. 1940-41.
2. Clayton, J. S. A study of the "bitter principle" in *Melilotus* species. M.Sc. Thesis, University of Saskatchewan, Saskatoon, Sask. 1934.
3. Clayton, J. S. and Larmour, R. K. A comparative color test for coumarin and melilotic acid in *Melilotus* species. Can. J. Research, 13 : 89-100. 1935.
4. Duncan, I. J. and Dustman, R. B. Quantitative determination of coumarin in plant material. Ind. Eng. Chem. Anal. Ed. 6 : 210-213. 1934.
5. Duncan, I. J. and Dustman, R. B. Determination of coumarin in sweet clover. A comparison of the steam-distillation and alcoholic-extraction methods. Ind. Eng. Chem. Anal. Ed. 9 : 471-474. 1937.
6. Dworak, L. The selection of elite forms of *Melilotus albus* with low coumarin content. Herbage Abstr. 7 : 217. 1937.
7. Gelcinskaja, R. B. and Bordunova, M. A. Breeding coumarin-free sweet clover. Herbage Abstr. 6 : 116. 1936.
8. Kanewskaja, S. G. and Fedorowa, M. F. A quantitative determination of coumarin and of melilotic acid in *Melilotus officinalis*. Z. anal. Chem. 93 : 176-180. 1933.
9. Rinke, E. H. Inheritance of coumarin in sweet clover. J. Am. Soc. Agron. 37 : 635-642. 1945.
10. Roberts, W. L. and Link, K. P. A precise method for the determination of coumarin, melilotic acid and coumaric acid in plant tissue. J. Biol. Chem. 119 : 269-281. 1937.
11. Roberts, W. L. and Link, K. P. Determination of coumarin and melilotic acid. A rapid micromethod for determination in *Melilotus* seed and green tissue. Ind. Eng. Chem. 9 : 438-441. 1937.
12. Slatensek, J. M. Some causes for variation of coumarin content in sweet clover. J. Am. Soc. Agron. 39 : 596-605. 1947.
13. Slatensek, J. M. and Washburn, R. L. A rapid, fluorometric method for the determination of coumarin and related compounds in sweet clover. J. Am. Soc. Agron. 36. 704-708. 1944.
14. Stevenson, T. M. and Clayton, J. S. Investigations relative to the breeding of coumarin-free sweet clover, *Melilotus*. Can. J. Research, 14 : 153-165. 1936.
15. Suvorov, V. V. Practical advances in breeding sweet clover. Herbage Abstr. 7 : 299. 1937.
16. Ufer, M. A method useful from a breeding standpoint to the selection of low coumarin forms of sweet clover (*Melilotus*). Züchter, 12 : 255-258. 1934. (English translation).
17. White, W. J. and Horner, W. H. Investigations concerning the coumarin content of sweet clover. II. Sources of variation in tests for coumarin content. Sci. Agr. 21 : 29-35. 1940.
18. White, W. J., Savage, R. G., and Johnston, F. B. A slightly modified fluorometric method of testing for coumarin content in sweet clover. Sci. Agr. 32 : 278-280. 1952.

STUDIES IN AMERICAN ORCHIDS

II. THE GENUS BRACHIONIDIUM LINDL.[1]

By Leslie A. Garay[2]

Abstract

The genus *Brachionidium* is one of the most poorly defined taxa in the whole Vegetable Kingdom. The obscurity displayed in the type descriptions of *B. parvifolium*, *B. tuberculatum*, and *B. concolor* has prevented systematists from understanding the genus properly and from studying its position and relationship among the genera of *Pleurothallidinae*. The genus originally included the three above-named species. With the aid of photographs of types, recent collections have been classified. In addition to these, five new species are described, all from tropical America, viz. from the Andean Region. In addition to the complete revision of this genus, and its immediate relationship, special attention is given to all 20 genera of the subtribe *Pleurothallidinae*. The interrelation of these genera is examined and illustrated from a dynamic point of view.

Introduction

In the whole Vegetable Kingdom very few genera exhibit such a lack of definition as does the genus *Brachionidium*, a member of the *Pleurothallidinae* in the family *Orchidaceae*. In the type collection of *Restrepia parvifolia*, upon which the genus was founded, the most important structures are absent, viz. the column, the anther, and the pollinia. The structure of the column was well observed in *B. tuberculatum*, but very vaguely in *B. concolor*. Neither anthers nor pollinia were seen in any of the three species.

The imperfectness referred to becomes even greater when the investigation is narrowed down to the specific level. In *B. parvifolium* the shape of the lip is unknown; in *B. concolor* and *B. tuberculatum* nothing is said about the sepals and petals. The whole description of the latter two species consists of one printed line, characterizing the shape of the lip and leaf only.

Through the courtesy of the Director of the Kew Herbarium, England, I am in possession of excellent photographs of all these Lindleyan species which, beside the actual specimens, bear Lindley's sketches of various floral parts omitted in the type descriptions.

Recent explorations in the Andes brought forth additional new species as well as others referable to the obscure Lindleyan concepts. These collections make it possible to present a complete revision of the genus. In addition, loans were arranged from every large herbarium on this continent and from several important ones in Europe, from which, however, the results obtained were rather discouraging because in most cases the material was limited to a single specimen.

Except for *B. Dussii* and *B. serratum* for which the nomenclatorial types were destroyed during the Second World War, the study presented here is based on firsthand observations.

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The accompanying illustrations were drawn from the type specimens or from specimens compared with the types. Details of the floral parts were completed with the aid of camera lucida.

Discussion

The genus *Brachionidium* was established by Lindley (4) in 1859 with three species. How imperfectly this genus was delimited at that time has just been explained. For the following 34 years only this mosaic picture was known to science. In 1893 Rolfe (6) discovered *B. Sherringii*, which he received in excellent condition. With the aid of this new addition to the genus, the exact shape of the column as well as the number of the pollinia were finally determined. Since that time eight taxa have been proposed by various students of Orchidology. With the exception of *B. Valerioi*, all were based on a single collection, and very often on a single specimen. Two species, viz. *B. brevicaudatum* (7) and *B. longicaudatum* (1), were discovered during scientific expeditions to Venezuela – in Mt. Roraima and Mt. Duida respectively.

All *Brachionidium* species are known to grow on the ground, and often deeply embedded among various mosses and selaginellas. The primary stem or rhizome is elongate, very slender in most of the species, rather thick and semiligneous in *B. parvifolium*, covered with scarious, tubular sheaths which are oblique at the apex where the prominent midrib is extended into a mucronate tip. The secondary stems are borne a considerable distance apart, very short, completely enclosed by scarious, imbricating sheaths similar to the ones observable on the rhizome, and bearing a single leaf at the tip. The leaves are of papery or semicoriaceous texture, very shortly petioled, and prominently veined at least in dry condition. The inflorescence is reduced to a single flower and borne on a more or less elongated peduncle, which originates from the joint of the secondary stem and leaf. The sepals and petals are often caudate, similar in size and shape; the lateral sepals are united up to the bifid tip. The fleshy, thickened lip is very minute, several times shorter than the sepals and petals in every species except in *B. Dussii*, where it is half as long as the lateral sepals. In natural position the lip is porrect and appressed to the front of the column. The column is short and thick without a distinct foot, though it may be dilated towards its base where the movable lip is jointed with it. The clinandrium is very prominent and laterally extended into two arm-like structures. The anther is two-celled, each cell containing three clavate or elongate-pyriform pollinia which are unequal in length.

The species of *Brachionidium* are found in tropical rain-forests at 2000 meters or more above sea level. It is hard to present a definite picture of the general distribution because, as it was stated, the material at hand is very limited. *Brachionidium Sherringii* seems to be the most common species in the genus and is of wide distribution. It is known to occur abundantly throughout the West Indies. *Brachionidium parvum*, which is also described

from the West Indies, is now known to be present on the mainland of South America, in Venezuela. The rest of the species, as it appears, have a local distribution. Two species, viz. *B. pusillum* and *B. Valerioi*, are of Central American origin, while the remaining ones are native to the Andes.

Relationships

When an attempt is made to show the natural position of a certain genus or group, it may be approached in two different ways. The first method, in which the viewer indicates how one particular genus or group has evolved from another in a straight line of descent, is called in Engler's sense the "Natural Classification", which, I believe, is a static and often an improbable one. The other view is one in which the relationship of a certain genus or group is examined in a three-dimensional perspective, determining its position on the basis of interrelated characteristics, in other words, presenting it in a dynamic way. Metaphorically, this latter view is similar to the arrangement of the Universe, in which, if we were to examine the position of our Globe, we would have to study its relation to our Solar System and compare the relation of our Solar System with the Galaxies, etc. In doing so, we are more likely to come closer to the actual truth.

In order to investigate the position of *Brachionidium* in such a dynamic system, we must determine whether or not this genus is a good and natural one. This examination can be executed only in comparing it with the other members of the *Pleurothallidinae* as well as with similar cases in the whole family.

Every taxonomist knows how hopelessly opinions differ regarding the delimitation of genera. The term genus, as it is employed today, is a matter of taste and convenience. However, we all may agree that the genus is the manifestation of various interrelated characteristics, common to, and partially present in each of its members.

I am continually searching for a criterion which may be employed with the maximum certainty in generic delimitation. While studying all generic descriptions in the *Orchidaceae*, I found that a given criterion, in one case, has a first degree importance, and in another case, it becomes a trivial matter only. The following examples will serve to substantiate this point of view: The column may be footless or extended into a foot in the genus *Dendrobium*. The same character separates *Maxillaria* and *Mormolyca* from each other.—The lateral sepals may or may not be extended into a sepaline-tube in the genus *Spiranthes*, and the same criterion is a differentiating one between *Broughtonia* and *Laeliopsis*.—The inflorescence may be one- or several-flowered in the genus *Oncidium*. On the other hand, *Lycaste* and *Xylobium* are kept separate on this very same basis. Similar cases could be cited endlessly. Naturally, these are sample characters which by themselves have no bearing on the delimitation of a genus.

Since the value of an individual character varies according to its position and employment, I submit that the only reliable criterion (in the orchids)

which definitely reflects on the interrelation of various kinds of organisms is the number of pollinia. This, of course, is not a new theory. It has been observed by all taxonomists of the past and present as an unwritten law.

However, in our times, we find some botanists who also consider the number of pollinia to be of trivial importance. I cannot resist calling to their attention Nietzsche's famous declaration: "Umwertung aller Werte", and this will become evident later.

The group *Pleurothallidinae* may be characterized as follows (8): The inflorescence is normally terminal (in one species, *P. Johnsonii*, it may be terminal or lateral). The labellum is always articulated with the base of the column. The viscid disk (when present) arises from the apex of the pollinia, is commonly irregular, but sometimes rudimentary or absent. The ovary is manifestly articulated with the pedicel. The pedicels are always persistent. The stem is slender, rigid, sometimes very abbreviated, and always unifoliate.

We find that the genera assigned here may have two, four, six, or eight pollinia. I am not in the position to state anything about the genetical conditions present among these genera, since none of them has been studied from a cytological point of view. Also, I can not possibly say that this genus is derived from that one or that from another. While attempting to establish a dichotomous evolutionary line, the more I studied the genera the more deeply I was convinced that such an evolutionary line does not exist in nature, because they are all interrelated like the meshes of a large net.

As it was said, the genus is the manifestation of various interrelated characters. In selecting the number of pollinia as the No. 1 criterion, we have four distinct groups with the following genera referable to them.

Group I.—Pollinia two

Acostaea Schltr.; *Crocodeilanthe* Rchb.f.; *Cryptophoranthus* Rodr.; *Lepanthes* Sw.; *Lepanthopsis* Ames; *Masdevallia* Ruiz & Pav.; *Physosiphon* Lindl.; *Physothallis* Garay; *Platystele* Schltr.; *Pleurothallis* R. Br.; *Porroglossum* Schltr.; *Scaphosepalum* Pfitz.; *Stelis* Sw.

Group II.—Pollinia four

Barbosella Schltr.; *Restrepia* H.B.K.; *Restrepiella* Garay (ined.).

Group III.—Pollinia six

Brachionidium Lindl.

Group IV.—Pollinia eight

Octomeria R. Br.; *Pleurothallopsis* Campos-Porto & Brade; *Yolanda* Hoehne.

I will attempt to show the interrelation of these genera, and the position of *Brachionidium* among them, based on various factors, and at the same time illustrate them on the attached diagram (see Fig. 1). After examining this diagram someone may say that, if the genera of the *Pleurothallidinae* are so much interrelated, there is no need to keep these taxa separate since such interrelation may indicate the presence of one well-advanced natural community. The student who entertains this view may be right, and future experimentation in bio- and cyto-taxonomic fields may reveal the necessity of such mass-unification, though I am very skeptical.

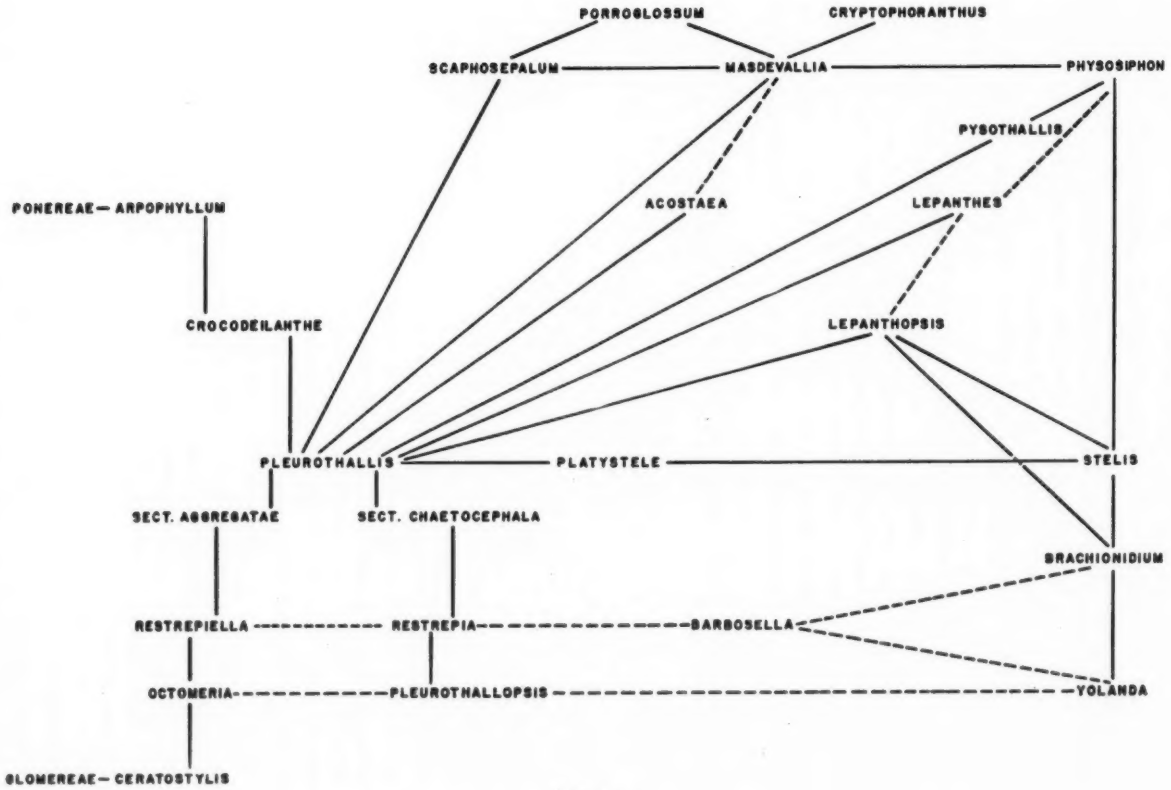
PORROGLOSSUM
CRYPTOPHORANTHUS
SCAPHOSEPALUM
MASDEVALLIA
PHYSOSIPHON
PYSOTHALLIS
PONEREAE — ARPOPHYLLUM
ACOSTAEA
LEPANTHES
CROCODEILANTHE
LEPANTHOPSIS
PLEUROTHALLIS
PLATYSTELE
STELIS
SECT. AGGREGATAE
SECT. CHAETOCEPHALA
BRACHIONIDIUM
RESTREPIELLA
RESTREPIA
BARBOSELLA
OCTOMERIA
PLEUROTHALLOPSIS
YOLANDA
GLOMEREAE — CERATOSTYLIS

FIG. 1.

Furthermore, the presence of the so-called "borderline-species" in various genera is certainly not an indication that through one or two such links a unification may be justified. On the contrary, the borderline-species are the only possible means by which the relationship between two different genera becomes apparent. To illustrate this, the reader is referred to Williams' paper (10) where he unites the genera *Laelia* and *Schomburgkia* on the presence of a single connective species, viz. *Laelia superbiens*.

Before going into proper explanation of the relationships of the *Pleurothallidinae*, I shall mention another article by Williams (9) in which the union of the genera *Pleurothallis* and *Restrepia* is proposed. He acknowledges that, since the genus *Restrepia* was established, every student of orchidology accepted it as being a well-defined one with the exception of George Bentham, the "master of generic definition" (page 141). Five pages later he says: "Bentham and Hooker in the Genera Plantarum so described *Coelia* that the characters of no plant which I know will fit it". It is hard to understand, how a "master of generic definition" can be quoted so diversely. Williams continues that the only distinguishing character is the number of pollinia, since taking into account the rest of the characteristics, they are all duplicated in *Pleurothallis*. He cites *P. ophiocephala* as having four pollinia, and it is not a *Restrepia*. Definitely, *P. ophiocephala* has four pollinia, so do *P. caliensis*, *P. tubulosa*, *P. pilosissima*, *P. hirsutissima*, and *P. ujarensis*. They all form a very natural group, or better, a genus, which exhibits the same degree of distinctive characters as does the genus *Octomeria*.

In *Octomeria* and *Pleurothallopsis* besides the number of pollinia every character is duplicated in *Pleurothallis*. Being so definite in proposing the union of *Restrepia* with *Pleurothallis*, Williams should have also given some consideration to *Octomeria* and *Pleurothallopsis*. The systematist who undertakes to carry out Williams' idea may find it extremely difficult to uphold any of the 20 genera of the *Pleurothallidinae*. It should be mentioned that a similar action like this was once employed by Reichenbach (5) when he united *Brassavola*, *Laelia*, and *Schomburgkia* with the genus *Bletia*. This treatment never was accepted by taxonomists, including Williams. Such radical action undoubtedly would loosen the intricate balance of the whole orchid family.

Since genera and groups of genera are interrelated, it is impossible to delimit a genus in such a manner that no character involved would be duplicated in another one. On the other hand, if one attempts to unite everything because of certain similarities, we will come to the point where we will have only one family, one genus, and one species called orchid.

As it has been mentioned, Williams proposed to unite *Schomburgkia* with *Laelia* based on the presence on an intermediate species. But if the number of pollinia is of no value, as he tried to show in the case *Restrepia* versus *Pleurothallis*, he should have proposed to unite *Cattleya* and *Laelia*, the only differentiating character of which is the number of pollinia. However, such a proposal would have met with a general disapproval by horticulturists and growers, as well as by systematists.

From what has just been said, it is evident that the number of pollinia is an important contributing factor in generic delimitation and especially in orchid systematics.

In returning to our main thesis, we find that the genus *Brachionidium*, the only one with six pollinia, occupies an important position in the perplexing system of *Pleurothallidinae*. Its footless column is short and extended laterally into two arm-like structures which are staminoid and sterile. The sepals and petals are of the same shape and size. The genus *Yolanda* exhibits the same characteristics, but it possesses eight pollinia. The striking homology between these two groups is undisputable, still they should not be united because in addition to the difference in the number of pollinia, the latter taxon is well separated geographically as well as ecologically. The bibrachiate and footless column is also a feature of *Stelis* and *Lepanthopsis*, but in these taxa the arms are fertile and accommodate the separate stigmas. In *Brachionidium* and *Yolanda* the sepals and petals are of the same shape and size, while in *Stelis* and *Lepanthopsis* they are of a very different nature.

On the other hand, the general habit, i.e. the creeping rhizome and the single-flowered inflorescence of *Brachionidium*, indicates a relationship with the genera *Pleurothallis* and *Barbosella*. However, in these two genera the column is extended into a foot and the anthers contain two and four pollinia respectively. The immediate relationship between them is quite apparent.

The group with eight pollinia includes in addition to *Yolanda* the genera *Octomeria* and *Pleurothallopsis*, but the connection is limited to this criterion only. While *Yolanda* is closely related to *Brachionidium*, *Octomeria* and *Pleurothallopsis* appear to have originated directly from *Pleurothallis*, though the separation must have taken place independently and the development on a parallel line. To these interrelated taxa belong *Restrepiella* and *Restrepia*, both with four pollinia. *Octomeria* and *Restrepiella* are entirely identical in general habit with the section Aggregatae of *Pleurothallis*, while *Restrepia* and *Pleurothallopsis* are homologues of the section Chaetocephala of the same genus. The peduncle is very much elongated and uniflowered in these taxa, and there is also some resemblance in the structure of the flowers, i.e. the petals terminate in a thickened clavate tip. At the same time, the shape of the lip in *Pleurothallopsis* and *Octomeria* is nearly identical.

The genus *Octomeria* seems to occupy a key position in this interrelated complex, because it indicates a connection between the subtribes *Pleurothallidinae* and *Glomereae* through the genus *Ceratostylis* (cf. *C. gigas*).

In the genus *Stelis* we have several borderline-species which, on one hand, indicate a elationship with *Pleurothallis* and, on the other hand, with the genus *Physosiphon*. In these three genera usually no deviation can be observed in the general growths aspect. In *Stelis oxypetala* all characters are typical for the genus with the exception of the receptive stigmas which are confluent into one body, and thus identically formed with that of *Pleurothallis*.

Stelis Dialissa, as it was pointed out before (2), is very different from the rest of the Stelides but the structure of its column maintains it in the genus. This is the only species where the three sepals are connate with each other more than half of their entire length, giving the calyx an urceolate appearance. The urceolate calyx is one of the main distinguishing features of *Physosiphon* and *Masdevallia*. The structure of the column in these two taxa indicates, on one hand, a definite connection with *Pleurothallis*, while on the other hand, the lepanthiform sheaths of *Physosiphon obliquipetalus* show a remote relation with the genus *Lepanthes*. This latter criterion further joins *Lepanthes* with *Pleurothallis*. The lip in the genus *Lepanthes* is highly adnate to the column, while in the genus *Pleurothallis* it is articulated with the column foot.

The urceolate calyx of *Physosiphon* offers a direct link with the genus *Masdevallia* and its allies. The relationship of *Masdevallia*, *Scaphosepalum*, and *Cryptophoranthus* has been discussed by Kraenzlin (3). It does not seem to be necessary, therefore, to repeat his interpretation, which is essentially the same as mine.

There is only one genus which has not been discussed directly, viz. *Pleurothallis*. This taxon seems to be the most prolific one, because in some respects it can be connected with every genus in the whole subtribe. This was shown in several cases. *Pleurothallis* encompasses about 1000 different species which offer a wide range of variation both in the floral structures and in the general appearance. However, *Pleurothallis Crocodeilanthe* exhibits unique features. The column in this species is footless, and the lip is firmly adnate to the base. In the other members of this genus these characteristics are of opposite nature. As it appears, *P. Crocodeilanthe* (originally proposed as a distinct genus) links the *Pleurothallidinae* with the subtribe *Ponereae* where the relationship with *Arpophyllum alpinum* is indicated by the habit of growth.

It is always difficult to describe a large system especially when it is similar to an intricate net. Should one loosen or take away one of the meshes, the whole net will collapse into an unrecognizable chaos. Nature itself is dynamic—consisting of organisms with dynamic potentialities which, as has been demonstrated, can only maintain their balance by mutual interrelation.

Acknowledgments

To The National Research Council of Canada, I wish to express my sincere thanks for the provided grant which made it possible to complete this study. To Sir Edward Salisbury, Director of the Kew Herbarium, England, I am deeply indebted for providing excellent photographs of the Lindleyan types, and to Dr. Charles Schweinfurth of Harvard University, Boston, Mass., U.S.A., for his generous gifts of type material of species described by him, I owe many thanks.

Thanks are due also to the Curators of the listed Herbaria for their co-operation, and for the permission to study their material.

Abbreviations

The abbreviations used in the main body of the monograph after each cited specimen are adapted from the Index Herbariorum as follows:

AMES: Orchid Herbarium of Oakes Ames, Botanical Museum, Harvard University, U.S.A.

F: Chicago Natural History Museum, U.S.A.

G: Conservatoire et Jardin Botaniques, Genève, Switzerland.

K: The Herbarium, Royal Botanic Gardens, Kew, England.

LD: Botanical Museum and Herbarium, Lund, Sweden.

NY: New York Botanical Garden, U.S.A.

P: Museum National d'Histoire Naturelle, Laboratoire de Phanerogámie, Paris, France.

S: Naturhistoriska Riksmuseet, Stockholm, Sweden.

US: United States National Museum, Smithsonian Institution, Washington, U.S.A.

W: Naturhistorisches Museum, Wien, Austria.

Brachionidium Lindley

Folia Orchidacea *Brachionidium* page 1, January 17, 1859; Bentham & Hooker, Genera Plant. 3 : 491. 1883; Pfitzer *in* Engler & Prantl, Die Natürl. Pflanzenfam. 2, Pt. 6 : 140. 1889; Cogniaux *in* Urban Symb. Antill. 6 : 450. 1910; Fawcett & Rendle, Fl. Jamaica, 1 : 76. 1910; Stehlé, Fl. Descript. Antill. Franç. 1 : 215. 1939; Ames *in* Field Museum Nat. History. Botan. Ser. 18, Pt. 1 : 199. 1937; Pittier, Cat. Fl. Venez. 1 : 204. 1945; Foldats *in* Anales Univ. Central Venezuela, 23 : 278. 1953; Hodge *in* Am. Orchid Soc. Bull. 23 : 41. 1954.

Sepala et petala inter se conformia, in acumen vel caudam longissimam producta; sepala lateralia saepius altius connata. Labellum indivisum seu trilobum, cum basi columnae articulatum, planum vel sigmoideum, satis crassum. Columna apoda, erecta, crassiuscula, brevis vel brevissima. Clinandrium 3-lobum, lobis lateralibus seu brachiis maximis, carnosulis, falcatis. Rostellum planum, late deltoideo-triangulum. Anthera terminalis, opercularis, incumbens. Pollinia 6, lineari-clavata vel elongato-pyriformia, apice visco parco cohaerentia. Capsula oblonga vel oblongo-ovoidea, obtuse trigona.

Herbae epiphyticae. Caulis primarius elongatus, scandens, simplex vel saepe ramosus, vaginis scariosis vestitus. Caules secundarii brevissimi vel ulli, monophylli. Folium subcoriaceum vel papyraceum, brevissime petiolatum. Pedunculus uniflorus. Flos mediocris vel majusculus.

Typus: *Restrepia parvifolia* Lindl.

Clavis Specierum[3]

1. Sepala petalaque acuminatissima vel in caudam longissimam producta 2
1*a*. Sepala petalaque acuta vel in acumen brevissimum producta 9
2. Planta fruticosa, erecta; folia 4 cm. longa ..... *B. parvifolium* Lindl.
2*a*. Planta herbacea, prostrata; folia minora 3
3. Labellum sigmoideum ..... *B. parvum* Cogn.
3*a*. Labellum planum 4
4. Apex sepalorum petalorumque acuminatissima nec caudata ..... *B. floribundum* Garay
4*a*. Apex sepalorum petalorumque proprie caudata 5
5. Flores 12 mm. in diametro ..... *B. pusillum* A. & S.
5*a*. Flores multo majores 6
6. Folia obovata, sepala ciliolata ..... *B. phalangiferum* Garay
6*a*. Folia elliptica vel elliptico-oblonga, sepala lateralia eciliata 7
7. Petala ciliata 8
7*a*. Petala eciliata ..... *B. meridense* Garay
8. Labellum antice denticulatum ..... *B. Valerioi* A. & S.
8*a*. Labellum antice integrum ..... *B. Sherringii* Rolfe
9. Folia conduplicata ..... *B. tetrapetalum* Schltr.
9*a*. Folia plana 10
10. Petala dense serrata ..... *B. serratum* Schltr.
10*a*. Petala integerrima 11
11. Labellum sepalorum lateralium dimidium aequans ..... *B. Dussii* Cogn.
11*a*. Labellum quam sepala lateralia multo minus 12
12. Sepala glaberrima 13
12*a*. Sepala ciliolata 15
13. Labellum triolobum ..... *B. brevicaudatum* Rolfe
13*a*. Labellum integerrimum 14
14. Sepala lateralia usque ad apicem connata, 4-nervia; labellum ecallosum ..... *B. concolor* Lindl.
14*a*. Sepala lateralia antice bifida, 6-nervia; labellum calliferum ..... *B. simplex* Garay
15. Labellum rhombeum, antice obtusatum ..... *B. tuberculatum* Lindl.
15*a*. Labellum cordatum, antice acutum ..... *B. ecuadorense* Garay

Descriptiones

Brachionidium brevicaudatum Rolfe *in* Trans. Linnean Soc. Botan. London, Ser. 2, Botany, 6 : 59. 1901; Cogniaux *in* Bull. soc. roy. botan. Belg. 43 : 320. 1906; Pittier, Cat. Fl. Venez. 1 : 204. 1945; Foldats *in* Anales Univ. Central Venezuela, 23 : 278. 1953. Fig. 4, D

Rhizomate prorepenti, ramuloso, cataphyllis membranaceis, scariosis vestito; caulibus secundariis brevissimis, vaginis scariosis, brevissime mucronatis omnino obtectis, 4–5 mm. longis, monophyllis; folio ovato-lanceolato, brevissime tricuspidato, acuto, basi in petiolum 2–3 mm. longum angustato, subcoriaceo, cum petiolo 1.7–2.3 cm. longo, 4–6 mm. lato; pedunculo filiformi, in medio univaginato, 2.7 cm. longo, unifloro; sepalo postico late ovato, apice in acumen abrupte producto, 3-nervio, 6–7 mm. longo, 4 mm. lato; sepalis lateralibus usque ad apicem connatis, sepalo postico simillimis, 4-nerviis, 6–7 mm. longis, 5 mm. latis; petalis ovatis, brevissime acuminatis, 3-nerviis, margine subtiliter ciliolatis, sepalis paulo brevioribus; labello trilobo, lobis lateralibus falcato-oblongis, subobtusis, lobo intermedio reflexo, late ovato-rotundato; columna humili, crassa.

[3]*This is an artificial key to the species. No attempt has been made to show the natural relationship.*

British Guiana: Mount Roraima Expedition. Roraima Range. Alt. 3500 ft. Coll. *F. V. McConnel & J. J. Quelch No. 705*, type (K).

Venezuela: Estado Bolivar; Sororopan-tepui, on ridge just below summit. Alt. 2225–2235 meters. Coll. *J. A. Steyermark No. 60947* (AMES, F).

This species is very closely related to *B. tuberculatum* Lindl.

Brachionidium concolor Lindl. Folia Orchid. Brachionid, p. 1, No. 2, 1859. Fig. 3, L–M

Caulibus primariis scandentibus, elongatis, vaginis scariosis, mucronatis obtectis; caulibus secundariis 1.5–3.5 cm. distantibus, satis abbreviatis, 9–11 mm. longis, vaginis tubulosis, scariosis omnino obtectis, apice monophyllis; folio ovato-lanceolato, acuto vel brevissime acuminato, basi abrupte in petiolum 4–5 mm. longum attenuato, usque ad 2.4 cm. longo, 5–7 mm. lato; pedunculo erecto, filiformi, in medio univaginato, usque ad 2.4 cm. longo, unifloro; sepalo postico ovato, concavo, 3-nervio, apice in acumen brevissimum producto, 9–10 mm. longo; sepalis lateralibus usque ad apicem connatis, valde concavis, late ovatis, brevissime acuminatis, 4-nerviis, 9–10 mm. longis, glabris; petalis obliquis, ovato-lanceolatis, apice brevissime caudatis, 6–7 mm. longis; labello e cuneata basi subrhombeo vel lunato, antice rotundato, lateraliter utrinque dente late triangulo, recurvo donato; disco 3-nervio, basin ecalloso; columna humili, satis crassa; brachiis prominentibus; capsula ovata, 1.3 cm. longa.

Bolivia: sine loco speciali. Coll. *Bridges s.n.* in 1846, type (K).

This description was amplified by the photograph of the type preserved in the author's herbarium.

Brachionidium Dussii Cogn. *in* Urban Symb. Antill. 6 : 451. 1910; Stehlé, Fl. Descript. Antill. Franç. 1 : 220. 1939

Rhizomate erecto, brevi, satis gracili; caulibus secundariis erectis, brevissimis, vaginis paucis, scariosis vestitis, 3–6 mm. longis, monophyllis; folio erecto-patulo, breviuscule petiolato, elliptico-oblongo, 1–2 cm. longo, 4–6 mm. lato; pedunculo solitario, filiformi, folio subduplo longiore, 2–3 cm. longo; sepalo postico ovato, breviter acuminato, 3-nervio, margine ciliato, 6 mm. longo, 3–3.5 mm. lato; sepalis lateralibus usque ad apicem connatis, ovatis, breviter obtuseque apiculatis, 4-nerviis, margine ciliolatis, 6 mm. longis, 3.5–4 mm. latis; petalis anguste ovatis, acutis vel subacuminatis, 3-nerviis, margine ciliolatis, 6 mm. longis, 2.5–3 mm. latis; labello ambitu late rhombeo, distincte trilobato, lobis lateralibus triangularibus, lobo intermedio leviter producto, 3 mm. longo et lato; disco nudo vel obscure calloso; columna humili, 1.5 mm. longa; capsula leviter oblonga, acutiuscula, trigona, 7 mm. longa, 3 mm. crassa.

Guadeloupe: Bains-Jaunes. Coll. *P. Duss No. 4149*, type.

Brachionidium ecuadorense Garay, sp. nov. Fig. 2, I–M

Rhizomate elongato, repenti, filiformi, vaginis scariosis, mucronatis remote vestito; caulibus secundariis inter se valde distantibus (ad 4 cm.), erectis vel

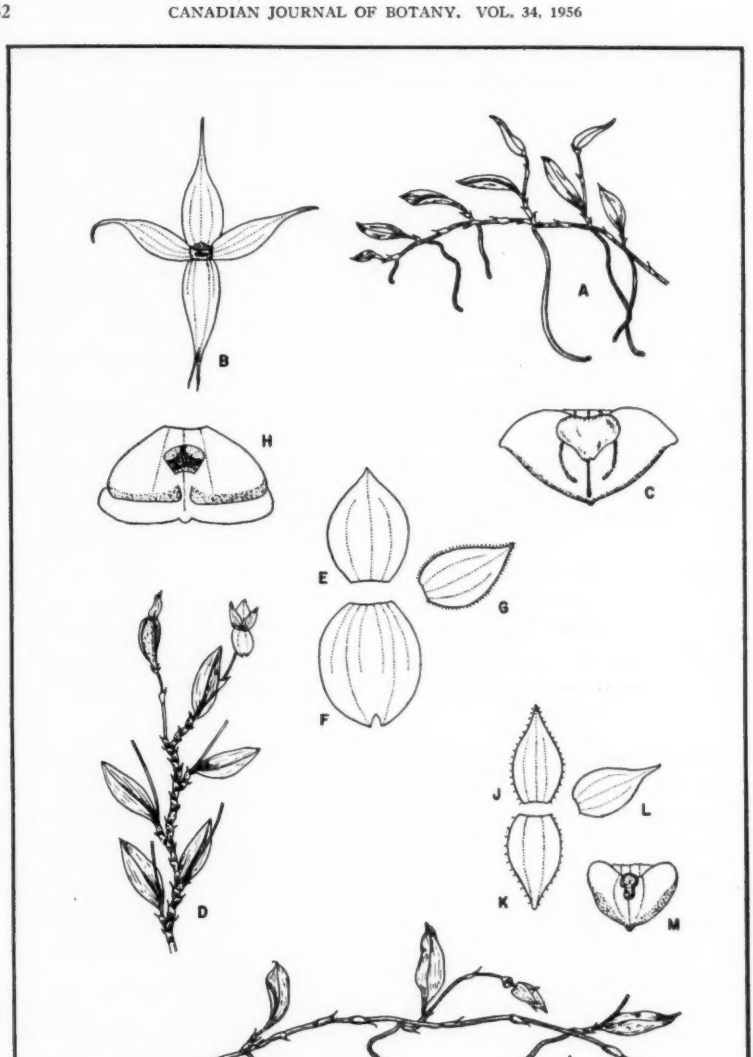

FIG. 2. A–C. *Brachionidium meridense* Garay. A. General habit × ⅔. B. Expanded flower × 2. C. Lip × 7. D–H. *Brachionidium simplex* Garay. D. General habit × ⅔. E. Dorsal sepal × 2.5. F. Lateral sepals × 2.5. G. Petal × 2.5. H. Lip × 7. I–M. *Brachionidium ecuadorense* Garay. I. General habit × ⅔. J. Dorsal sepal × 1.5. K. Lateral sepals × 1.5. L. Petal × 1.5. M. Lip × 5.

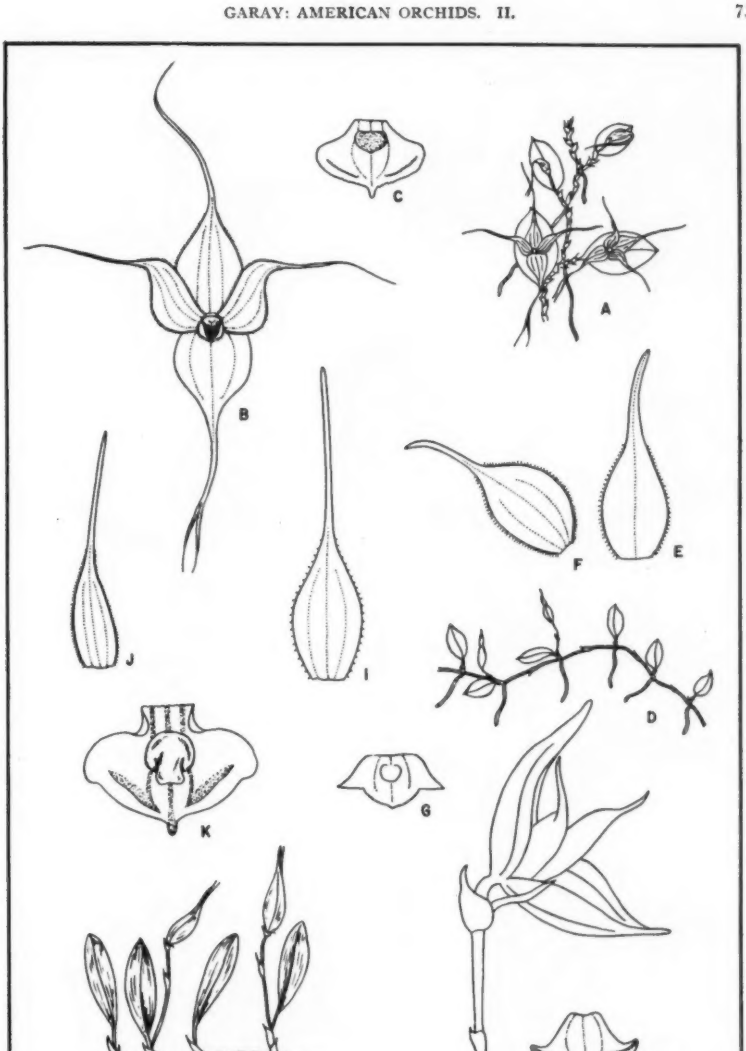

FIG. 3. A–C. *Brachionidium Valerioi* A. & S. A. General habit × ⅔. B. Expanded flower × 1.5. C. Lip × 10. D–G. *Brachionidium pusillum* A. & S. D. General habit × ⅔. E. Dorsal sepal × 4. F. Petal × 6. G. Lip × 4. H–K. *Brachionidium phalangiferum* Garay. H. General habit × ⅔. I. Dorsal sepal × 2. J. Petal × 1.5. K. Lip × 10. L–M. *Brachionidium concolor* Ldl. L. Lateral view of the flower × 3 M. Lip × 4.

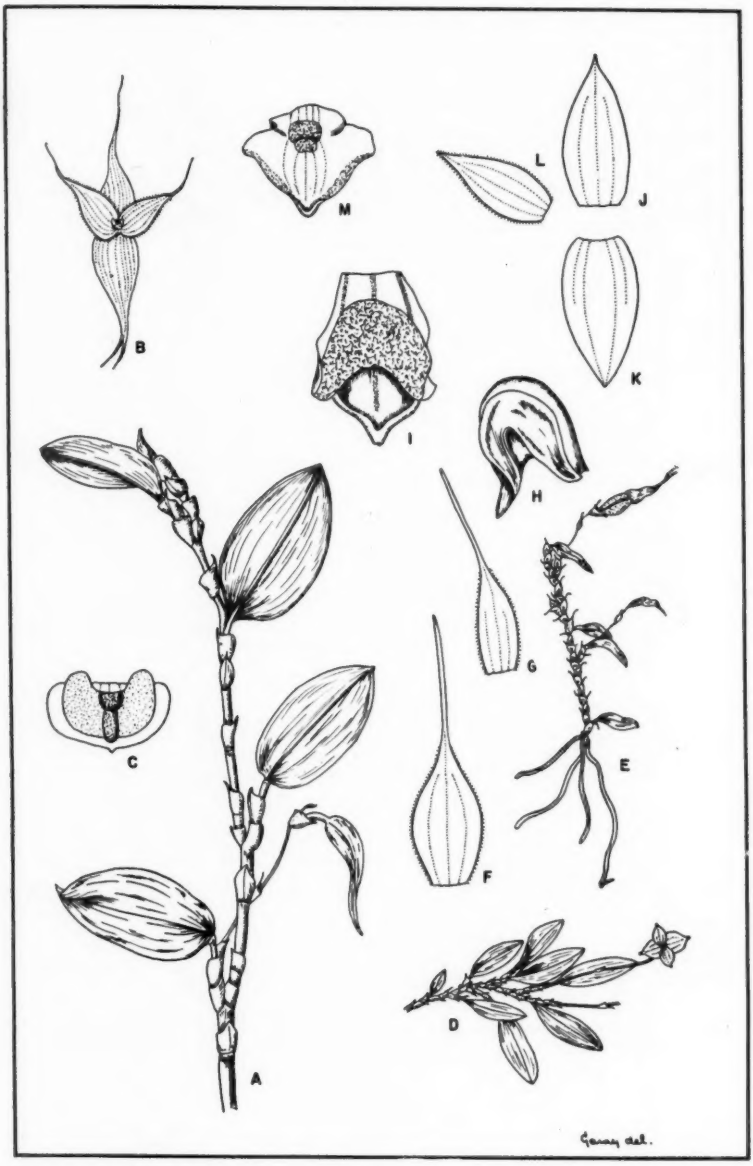

FIG. 4. A–C. *Brachionidium parvifolium* Ldl. A. General habit ⅔ natural size. B. Expanded flower ¾ natural size. C. Lip × 4. D. *Brachionidium brevicaudatum* Rolfe × ⅔. E–I. *Brachionidium parvum* Cogn. E. General habit × ⅔. F. Dorsal sepal × 4. G. Petal × 4. H. Lateral view of the lip × 25. I. Expanded lip × 25. J–M. *Brachionidium tetrapetalum* Schltr. J. Dorsal sepal × 3. K. Lateral sepals × 3. L. Petal × 3. M. Lip × 8.

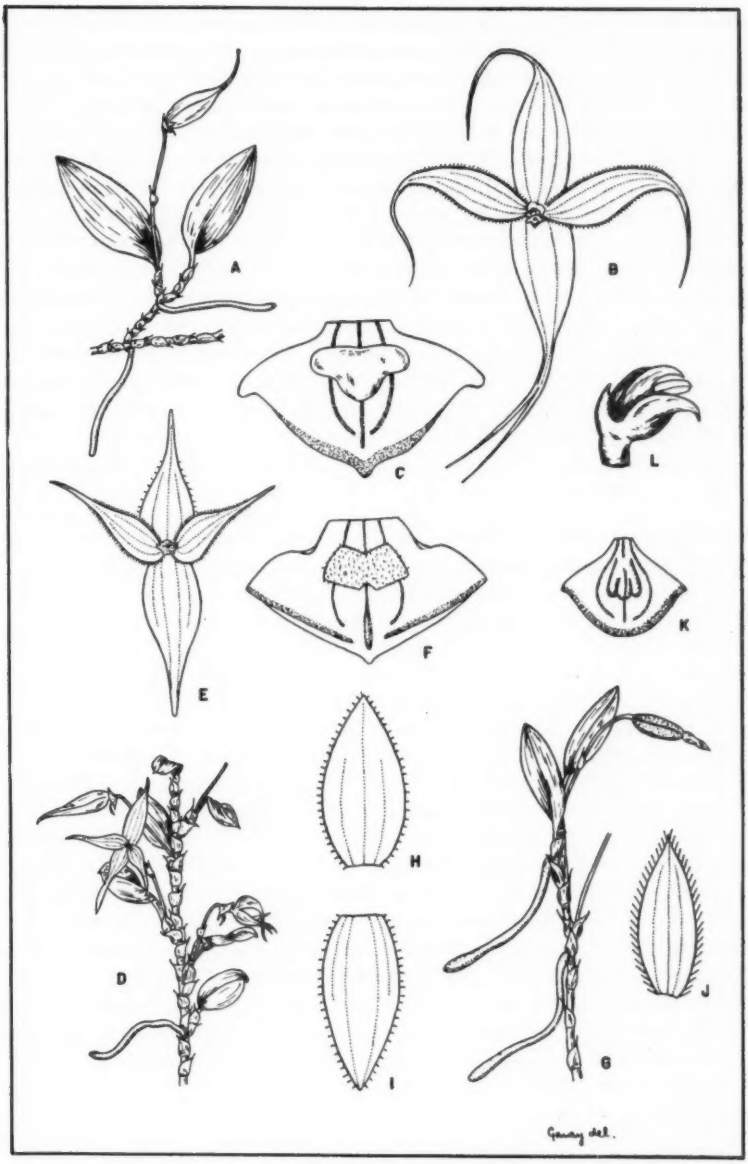

FIG. 5. A–C. *Brachionidium Sherringii* Rolfe. A. General habit × $\frac{3}{4}$. B. Expanded flower × 1.5. C. Lip × 12. D–F. *Brachionidium floribundum* Garay. D. General habit × $\frac{3}{4}$. E. Expanded flower × 1.5. F. Lip × 8. G–L. *Brachionidium tuberculatum* Lindl. G. General habit × $\frac{3}{4}$. H. Dorsal sepal × 3. I. Lateral sepals × 3. J. Petal × 3. K. Lip × 8. L. Side view of the column × 7.

suberectis, brevissimis, cum vaginis binis omnino obtectis, monophyllis, 4–5 mm. longis; folio semicoriaceo, ovato-oblongo, tridenticulato-mucronato, brevissime petiolato, 18–22 mm. longo, 5–7 mm. lato; pedunculo gracili, suberecto vel leviter arcuato, in medio univaginato, 20–25 mm. longo, unifloro; sepalis petalisque inter se simillimis, ut videtur purpurascentibus; sepalo postico late ovato-lanceolato, brevissime acuminato, 3-nervio, margine subtiliter ciliato, 9 mm. longo, 5 mm. lato; sepalis lateralibus usque ad apicem connatis, late ovato-lanceolatis acutis vel brevissime acuminatis, concavis, binerviis, margine ciliatis, 9 mm. longis, 4 mm. latis; petalis sepalis simillimis, 3-nerviis, margine densissime ciliatis, 8 mm. longis, 4 mm. latis; labello cordato, antice obtuse apiculato, margine incrassato, 2–2.5 mm. longo, 3 mm. lato; disco 3-nervio, tuberculo supra basin bipartito donato; columna humili, satis crassa.

Ecuador: Prov. Azuay: Cordillera Oriental, Sigsig. Alt. 2800–3200 meters. Coll. *F. C. Lehmann No. 7057*, type. Type in the herbarium of the New York Botanical Garden (NY).

This species seems to be closely related to *B. tuberculatum* but the form of the lip easily differentiates the two.

Brachionidium floribundum Garay, sp. nov. Fig. 5, D–F

Caulibus primariis scandentibus, cum vaginis scariosis, densissime imbricantibus omnino obtectis; caulibus secundariis ascendentibus, bivaginatis, ad 5 mm. longis, monophyllis; folio pergameneo, ovato, acuto, brevissime mucronato, basi in petiolum conduplicatum, 2–3 mm. longum abrupte angustato, 1.5–2 cm. longo, 7–10 mm. lato; pedunculo gracili, in medio univaginato, ad 2 cm. longo, unifloro; sepalo postico ovato-lanceolato, apice anguste acuminato nec caudato, 3-nervio, margine subtiliter ciliato, 15 mm. longo, 6 mm. lato; sepalis lateralibus usque ad apicem in valvam ovato-laneolatum, anguste acuminatam, apice brevissime bifidam connatis, 4-nerviis, margine integerrimis, 17 mm. longis, 7 mm. latis; petalis obliquis, ovato-lanceolatis, longe attenuatoque acuminatis, sic caudatis, 3-nerviis, margine dense ciliatis, 14 mm. longis, 4 mm. latis; labello subrhombeo, satis crasso, antice acuto et brevissime obtuseque apiculato, margine incrassato, 2.5 mm. longo, 5 mm. lato; disco 3-nervio, supra basin cum callo trapezoideo, antice inciso, densissime pubescenti ornato; columna satis crassa, leviter arcuata; brachiis acuminatis, leviter falcatis; rostello late triangulo, apiculato.

Venezuela: Estado Merida: Jaji. Alt. 2700 meters. April 28, 1949. Coll. *O. Renz No. 5253*, type. Between Tovar and Guaraque. Alt. 2400 meters. July 18, 1951. Coll. *O. Renz No. 7248* (Herb. Renz.). Type in the herbarium of Dr. O. Renz. Part of the type collection in the author's herbarium No. 3556.

Brachionidium meridense Garay, sp. nov. Fig. 2, A–C

Caulibus primariis filiformibus, longe repentibus, vaginis scariosis, satis adpressis remote vestitis; caulibus secundariis inter se 1.5–2 cm. distantibus, erectis, minutissimis vel ullis, vaginis binis omnino obtectis, 1–2 mm. longis.

monophyllis; folio ovato-lanceolato, utrinque attenuato, apice acuto, basi in petiolum brevissimum attenuato, 12–22 mm. longo, 3–7 mm. lato; pedunculo filiformi, in medio univaginato, 12–15 mm. longo, apice unifloro; sepalo postico ovato-lanceolato, apice caudato, 3-nervio, margine eciliato, 12 mm. longo, 4 mm. lato; sepalis lateralibus in valvam ovato-lanceolatam, apice caudatum, brevissime bifidam connatis, 4-nerviis, margine eciliatis, 12 mm. longis, 4 mm. latis; petalis leviter obliquis, lanceolatis, caudatis, 3-nerviis, margine eciliatis, 11 mm. longis, 3 mm. latis; labello subrhombeo, lateraliter utrinque angulato, antice subacuto et brevissime apiculato, margine incrassato, 2 mm. longo, 3 mm. lato; disco 3-nervio supra basin callifero; columna humili, brachiis satis prominentibus, acuminatis; capsula ovoidea, leviter angulata, 7 mm. longa.

Venezuela: Estado Trujillo. Between Bocono and La Cuchilla. Alt. 2800 meters. Nov. 3, 1951. Coll. *O. Renz No. 7507*, type. Type in the herbarium of Dr. O. Renz. Part of the type collection the author's herbarium No. 3554.

This new species appears to be related to *B. Valerioi*, but the latter has ciliolate sepals and a denticulate lip.

Brachionidium parvifolium Lindl. Folia Orchid. Brachionidium, p. 1, No. 1, 1859

Syn.: *Restrepia parvifolia* Lindl. Orch. Linden. p. 4, No. 18, 1846. Fig. 4, A–C

Frutex, erectus, ramosus, usque ad 40 cm. altus; caulibus primariis elongatis, satis crassis, semilignosis, 3 mm. crassis, vaginis dilatatis, dorso mucronatis, scariosis remote vestitis; caulibus secundariis suberectis vel ascendentibus, satis brevibus, vaginis binis, scariosis, imbricatis omnino obtectis, 1–1.5 cm. longis, monophyllis, folio ovato-lanceolato vel elliptico, acuto vel abrupte acuminato, basi in petiolum conduplicatum abrupte, attenuato, satis coriaceo, 3.5–4 cm. longo, 1–2 cm. lato; pedunculo erecto, tereti, in medio univaginato, 3–4 cm. longo, unifloro; sepalo postico ovato-lanceolato, longissime caudato, 7-nervio, margine eciliato, 3 cm. longo, 7 mm. lato; sepalis lateralibus connatis, ovato-lanceolatis, longissime caudatis, apice bifidis, 7-nerviis, margine eciliatis, 3.25 cm. longis, 8–10 mm. latis; petalis ovato-lanceolatis, longissime caudatis, leviter obliquis, 5-nerviis, margine distincte ciliolatis, 2.5–2.8 cm. longis, 5–7 mm. latis; labello carnosissimo, tenuiter marginato, ambitu suborbiculari, antice obtuso, in medio brevissime apiculato, 3 mm. longo, 5 mm. lato; disco basin verrucoso-tuberculato; columna minuta, crassa, 2 mm. alta; brachiis utrinque dentiformibus.

Colombia: Dept. of Tolima. On the slope of the Peak of Tolima. Coll. *Linden No. 1278*, type (K). Dept. of Cundinamarca; North of San Miguel. Alt. 2800 meters. Coll. *O Renz No. 3835* (in herb. Renz); ibidem Coll. *J. G. Hawkes & H. Garcia Barriga No. 40* (US). Territorio Caqueta: Santiago between Pasto and Mocoa. Coll. *F. C. Lehmann s.n.* (W). Dept. of Narino: Cordillera Oriental; Bordencillo, east of Pasto. Alt. 3000 meters.

Feb. 15, 1881. Coll. *F. C. Lehmann No. 344* (W). Intendencia del Choco: 10 km. west southwest of Carmen de Atrato, on ridge east of Río Girardol. Alt. 2000 meters. Coll. *F. R. Fosberg & E. L. Core No. 21522* (US).

Lehmann reports that this species has an appearance of a small shrub with erect branches up to 40 cm. tall.

Brachionidium parvum Cogn. *in* Fedde Rep. 6 : 307. 1909; *in* Urban Symb. Antill. 6 : 451. 1910

Syn.: *B. longicaudatum* Ames & Schweinf. *in* Bull. Torrey Botan. Club, 58 : 348. 1931; Pittier, Cat. Fl. Venez. 1 : 204. 1945; Foldats *in* Anales Univ. Central Venezuela, 23 : 278. 1953; *B. Sherringii* Rolfe var. *parvum* Stehlé, Fl. Descript. Antill. Franç. 1 : 219. 1939. Fig. 4, E–I

Rhizomate erecto, gracili, cum vaginis approximatis vel imbricatis, tubulosis, ad apicem caudato-mucronatis obtectis; caulibus secundariis erectis, minutissimis, per totam longitudinem cum vaginis binis, illis rhizomatis simillimis, circumdatis, 2–5 mm. longis; folio satis coriaceo, elliptico, brevissime petiolato, 7.5–15 mm. longo, 2–5 mm. lato; pedunculo solitario, filiformi, unifloro, folio multo longiore; bractea infundibuliformi-spathacea, apice truncata et brevissime apiculata; sepalis inter se simillimis, ovato-lanceolatis, longissime caudato-acuminatis, membranaceis, margine subtiliter ciliatis; sepalo postico 3-nervio, 9–10 mm. longo, 3–3.5 mm. lato; sepalis lateralibus in valvam apice minute bidentatam connatis, 4-nerviis, 10 mm. longis, 3–4 mm. latis; petalis sepalo dorsali simillimis, leviter obliquis, ovato-lanceolatis, caudato-acuminatis, 3-nerviis, margine subtiliter ciliatis, 6–8 mm. longis, 2–2.5 mm. latis; labello minuto, sigmoideo, parte basali subquadrato, parte apicali triangulari interdum lateraliter juxta plicam dente minuto instructo; disco 3-nervio, a basi usque ad medium callo incrassato, superne bilobo ornato; columna crassa, minutissima; brachiis patulis, anguste-triangularibus vel potius dentiformibus acutis.

Guadeloupe: La Soufriers. Coll. *P. Duss No. 3730*, type (NY); Grand Carbet. Coll. *Stehlé No. 1368* (NY), *No. 1369*, *No. 1372*.

Martinique: La Mont Pelée. Coll. *Hahn No. 1266* (G); Piton du Carbet. Coll. *Stehlé No. 3069;* Piton de Du Mause. Coll. *Stehlé No. 3344*.

Grenada: between Soulier and Azemar. Coll. *Broadway No. 268*, *No. 1830*.

Venezuela: Summit of Mount Duida. Coll. *G. H. H. Tate No. 682* (type of *B. longicaudatum* A. & S.) (NY).

The nomenclatorial type of both species, viz. *B. parvum* and *B. longicaudatum*, is to be found in the collection of the New York Botanical Garden. The examination of these did not reveal any substantial difference upon which a separation may be based. The reduction of *B. parvum* to varietal rank by Stehlé is unjustified. Apparently Stehlé did not see the type of *B. parvum*.

Brachionidium phalangiferum Garay, sp. nov. Fig. 3, H–K

Caulibus primariis longe repentibus, vaginis amplis, scariosis, imbricatis

omnino vestitis; caulibus secundariis erectis, bivaginatis, 4–5 mm. longis, unifoliatis; folio semicoriaceo, obovato, apice obtuso et dorso mucronato, basi in petiolum 5–7 mm. longum attenuato, cum petiolo 2–3 cm. longo, 6–8 mm. lato; pedunculo gracili, in medio univaginato, 1.5 cm. longo, unifloro; sepalo postico ovato-lanceolato, longissime caudato, 3-nervio, margine subtiliter ciliato, 2.5 cm. longo, 5 mm. lato; sepalis lateralibus in valvam ovato-lanceolatam, longissime caudatam, apice breviter bifidam connatis, 4-nerviis, margine subtiliter ciliolatis, 2.5 cm. longis, 7 mm. latis; petalis ovato-lanceolatis, longissime caudatis, 3-nerviis, margine densissime ciliatis, 2 cm. longis, 4 mm. latis; labello transverse subrhombeo, antice apiculato, margine integerrimo, 2 mm. longo, 3 mm. lato; disco 3-nervio, in medio cum callo subrotundo, antice truncato ornato; columna humili, crassa; brachiis leviter falcatis, obtusis; capsula oblonga, 1.5 cm. longa.

Venezuela: Estado Merida. Between Sta. Cruz and El Molino. Alt. 2400 meters. Nov. 12, 1949. Coll. *O. Renz No. 6109*, type. Type in the herbarium of Dr. O. Renz. Part of the type collection in the author's herbarium No. 3555.

Brachionidium pusillum Ames & Schweinf. *in* Ames, Sched. Orch. No. 10 : 52, 1930; Ames *in* Field Museum Nat. History. Botan. Ser. 18, pt. 1 : 199. 1937. Fig. 3, D–G

Rhizomate filiformi, elongato, repenti; caulibus secundariis minutissimis erectis in vaginis binis, scariosis inclusis, unifoliatis, 1 mm. longis; folio elliptico apice tridenticulato-mucronato, basi in petiolum minutissimum abrupte attenuato, usque ad 9 mm. longo, 4 mm. lato; inflorescentia uniflora; pedunculo filiformi usque ad 7 mm. longo; sepalo postico ovato-lanceolato, prominenter caudato, uninervio, 6.1 mm. longo, 2 mm. lato, margine ciliato; sepalis lateralibus usque ad apicem connatis, ovato-lanceolatis, caudatis, binerviis, 5.2 mm. longis, 1.6 mm. latis; petalis ovato-lanceolatis, caudato-acuminatis, 3-nerviis, leviter obliquis, 5.6 mm. longis, 2 mm. latis, margine minutissime ciliolatis; labello trilobo, lobis lateralibus triangulis, acutis, lobo intermedio rotundato, brevissime apiculato; disco 3-nervio in medio callo crasso, rotundato donato; toto labello 1.6–2 mm. longo, 3–4 mm. lato; columna brevissima, crassa, sursum dilatata; clinadrio lateraliter utrinque triangulo-unidentato.

Costa Rica: Province of Heredia, Yerba Buena, northeast of San Isidro. Coll. *P. C. Standley & J. Valerio No. 49068*, type (AMES, US); ibidem *No. 50250* (AMES, US).

Brachionidium serratum Schltr. *in* Fedde Rep. 9 : 164. 1911

Caulibus primariis plusminusve elongatis, vaginis arcte appressis obtectis; caulibus secundariis brevibus, teretibus, vix 0.5 cm. longis, unifoliatis; folio elliptico, obtusiusculo, inaequaliter bilobulato, coriaceo, 0.7–1.6 cm. longo, 3–4 mm. lato; pedunculo gracili, evaginato, unifloro; sepalo postico ovato-lanceolato, apiculato, dorso nervo medio carinato, margine integerrimo, 8 mm. longo, 4 mm. lato; sepalis lateralibus usque ad apicem in laminam ovatam, obtusiusculam connatis, margine integerrimis, 8.5 mm. longis, 5 mm. latis;

petalis oblique lanceolatis, acuminatis, margine dense serratis, sepalo intermedio subaequilongis; labello e basi quadrato-unguiculato, late rhombeo, breviter apiculato, angulis lateralibus obtusiusculis, in medio callo rotundato ornato; ovario cylindraceo, ca. 0.3 cm. longo.

Peru: Chochobamba. Coll. *Dr. Filomeno No. 1907*, type.

Brachionidium Sherringii Rolfe *in* Kew Bull. p. 4, 1893; Cogniaux *in* Urban Symb. Antill. 6 : 450. 1910; Fawcett & Rendle, Fl. Jamaica, 1 : 77. 1910; Stehlé, Fl. Descript. Antill. Franç. 1 : 215. 1939; Hodge *in* Am. Orchid Soc. Bull. 23 : 41. 1954

Syn.: *Brachionidium parvum* Cogn. p.pt. *in* Fedde Rep. 6 : 307. 1909. Fig. 5, A–C

Caulibus primariis elongatis, leviter ramosis, scandentibus, vaginis scariosis, mucronatis omnino vestitis; caulibus secundariis erectis vel ascendentibus, vaginis 2–3, imbricatis obtectis, 3–9 mm. longis, apice unifoliatis; folio elliptico-oblongo, apice acuto tridentato-mucronatoque, basi in petiolum conduplicatum, usque ad 6 mm. longum abrupte contracto, cum petiolo 1.5–3.5 cm. longo, 5–12 mm. lato; pedunculo erecto, gracili, in medio univaginato, unifloro, 2.5–4 cm. longo; sepalo postico ovato-lanceolato, 3-nervio, longissime caudato, 16–30 mm. longo, 3–5 mm. lato; sepalis lateralibus in valvam apice bifidam connatis, dorsali simillimis et fere aequimagnis, 4-nerviis; petalis ovato-lanceolatis, longissime caudatis, 3-nerviis, margine leviter ciliatis, 2.5 cm. longis, 2.5–4 mm. latis; labello breviter unguiculato, ambitu rhombeo, superne leviter trilobato, lobis lateralibus late triangularibus, semifalcatis, acutis, lobo intermedio late deltoideo, acuto; disco 3-nervio, supra basin calloso, toto labello 1.5–2 mm. longo et totidem lato; columna humili, brachiis falcato-ovatis, acutis.

Grenada: Top of Fedon's Camp. Coll. *R. V. Sherring s.n.*, type (K); ibidem Coll. *W. E. Broadway No. 972* (NY, US); Mount St. Catherine. Coll. *P. Beard No. 1310* (US).

Guadeloupe: Riviere Saint-Louis. Coll. *H. Stehlé No. 398* (NY); Ravine Longueteau. Coll. *H. Stehlé No. 2029* (US); Matouba Trois Rivieres. Coll. *P. Duss No. 3345* (AMES, NY, US); Savane aux Ananas. Coll. *H. Stehlé No. 429* (NY), *No. 1370* (NY); ibidem Coll. *P. Duss No. 3980* (NY), *No. 3971* (NY); Forêts au nord du Matéliane. Coll. *H. Stehlé No. 470* (NY); Entre Ravine Longueteau. Coll. *H. Stehlé No. 2518* (NY); Terace du Lac Flammarion. Coll. *H. Stehlé No. 1369* (NY); ibidem *No. 2470* (NY); Trace humide de la Citerne. Coll. *H. Stehlé No. 1371* (NY), *No. 1681* (NY).

Martinique: Pitons du Carbet, Piton du Morne-Vert. Coll. *H. Stehlé No. 2503* (NY), *No. 1372* (NY), *No. 1368* (NY); Mont Pelée. Coll. *P. Duss No. 4686* (NY); sine loco speciali. Coll. *Bélanger No. 766* (G), *No. 230* (P).

St. Kitt's: Mt. Misery. Coll. *M. L. Britton & J. F. Cowell No. 524* (NY, US).

Dominica: Morne Trois Pitons. Coll. *W. H. Hodge No. 398* (AMES, NY), *No. 1382* (NY); Mt. Drablatin. Coll. *F. E. Lloyd No. 908* (NY).

Jamaica: Mt. Moses. Coll. *W. Harris s.n.* (NY); St. Andrew, Mt. Horeb, Port Royal Mts. Coll. *G. R. Proctor No. 6847* (in herb. Inst. Jamaica).

Haiti: sine loco speciali. Coll. *Picarda s.n.* (ex Cogn. loc. cit.).

Brachionidium simplex Garay, sp. nov. Fig. 2, D–H

Rhizomate elongato, flexuoso, vaginis scariosis omnino obtecto; caulibus secundariis satis breviusculis, omnino vaginis scariosis parce evanidis vestitis, 4–5 mm. longis, monophyllis; folio plano, crasse coriaceo, ovato-oblongo, acuto vel brevissime acuminato, basi abrupte in petiolum canaliculatum attenuato, 1.7–2 cm. longo, 5–7 mm. lato; pedunculo filiformi, leviter arcuato, in medio univaginato, unifloro; sepalo postico ovato, acuto vel brevissime acuminato, ecaudato, 3-nervio, 7 mm. longo, 3.5 mm. lato; sepalis lateralibus in valvam subrotundam, apice brevissime bidentatam omnino connatis, 6-nerviis, glabris, 7 mm. longis, 5 mm. latis; petalis ovato-lanceolatis, acutis vel brevissime acuminatis, 3-nerviis, margine ciliatis, 6 mm. longis, 2.5 mm. latis; labello e cuneata basi dilatato, antice truncato, in medio sinuato cum apiculo obtuso interjecto; disco 3-nervio in medio callifero, lateraliter infra apicem transverse in lineam incrassatam producto, 2 mm. longo, 3 mm. lato; columna humili; brachiis abbreviatis; capsula ellipsoidea, leviter sulcata, ca. 1 cm. longa.

Ecuador: Quito. Coll. *Jameson s.n.*, type. Type in the author's herbarium No. 2817.

B. simplex is very similar to *B. brevicaudatum* Rolfe from which it differs in having a simple lip and ecaudate sepals.

Brachionidium tetrapetalum (Lehm. & Krzl.) Schltr. *in* Fedde Rep. Beih. 7 : 241. 1920

Syn.: *Pleurothallis tetrapetala* Lehm. & Krzl. *in* Engl. Bot. Jahrb. 26 : 446. 1899. Fig. 4, J–M

Caulibus primariis longe prorepentibus, ramulosis, cum vaginis scariosis omnino obtectis; caulibus secundariis minutissimis vel ullis, unifoliatis; folio conduplicato, acuto, basin versus in petiolum angustato, semicoriaceo, 1–1.5 cm. longo, 2–4 mm. lato; pedunculo gracili, leviter arcuato, in medio univaginato, usque ad 1.5 cm. longo, apice unifloro; sepalo postico late ovato-lanceolato, brevissime acuminato, 3-nervio, margine integro, 7 mm. longo, 3.5 mm. lato; sepalis lateralibus ovato-lanceolatis in valvam usque ad apicem connatis, acutis, 4-nerviis, margine integerrimis, 7 mm. longis, 3 mm. latis; petalis ovato-lanceolatis, apice attenuato-acuminatis, ecaudatis, 3-nerviis, margine subtiliter ciliatis, 6 mm. longis, 2.5 mm. latis; labello transverse rhombeo, antice acuto leviterque incrassato, 5-nervio, 2 mm. longo, 3 mm. lato; disco papilloso-tuberculato; columna humili; brachiis dentiformibus; capsula ovoidea, 5 mm. longa.

Colombia: Dept. of Narino. Cordillera Oriental; Bordencillo, east of Pasto. Alt. 3300 meters. Feb. 20, 1881. Coll. *F. C. Lehmann No. 347*, type (W).

Brachionidium tuberculatum Lindl. *in* Folia Orchid. Brachionidium, p. 1, No. 3. 1859. Fig. 5, G–L

Caulibus primariis longe repentibus, vaginis scariosis mucronatis vestitis; caulibus secundariis ascendentibus vel suberectis, vaginis tribus, illis in rhizomate observatis simillimis omnino obtectis, 8–10 mm. longis, apice monophyllis; folio ovato-oblongo, semicoriaceo, apice mucronato, basi in petiolum 4–5 mm. longum abrupte attenuato, cum petiolo 2–3 cm. longo, 5–7 mm. lato; pedunculo satis gracili, in medio univaginato, 2–2.5 cm. longo, unifloro; sepalis inter se simillimis, margine subtiliter ciliatis; sepalo postico ovato-lanceolato, apice in acumen brevissimum producto, 3-nervio, 7.25 mm. longo, 3.75 mm. lato; sepalis lateralibus in unum coalitis, valde concavis, apice acutis vel in acumen brevissimum productis, 4-nerviis, 7.5 mm. longis, 3.5 mm. latis; petalis sepalis conformibus, 3-nerviis, margine densissime ciliatis, 6.5 mm. longis, 3 mm. latis; labello e cuneata basi subrhombeo, antice obtuso, lateraliter utrinque obscure angulato, margine superiore incrassato, 2.5 mm. longo, 3.5 mm. lato; disco 3-nervio, nervo mediano supra basin cum callo vel tuberculo antice trilobato ornato; columna humili, crassa; brachiis late triangulis, antice recurvis; capsula ovoidea, leviter sulcata, 8–10 mm. longa.

Colombia: Paramo Cumbal. Coll. *H. Karsten s.n.* (W).

Venezuela: Prov. Merida. Alt. 8000 ft. Juni 1847. Coll. *Funck & Schlim No. 1558*, type (K, LD).

The type specimen in the Kew Herbarium indicates the original locality as "New Grenada", while an isotype in the Lund Herbarium clearly states Venezuela, Prov. Merida. The handwriting on both labels is identical.

Brachionidium Valerioi Ames & Schweinf. *in* Ames, Sched. Orch. No. 10 : 53. 1930; Ames *in* Field Museum Nat. History. Bot. Ser. 18, pt. 1 : 199. 1937. Fig. 3, A–C

Caulibus primariis longe repentibus, filiformibus, vaginis scariosis obtectis; caulibus secundariis ad 2.5 cm. distantibus, abbreviatis, 5 mm. longis, vaginis binis, infundibuliformibus, mucronatis omnino obtectis, unifoliatis; folio elliptico vel lanceolato-elliptico, acuto, basi brevissime petiolato, 1–2 cm. longo, 4–8 mm. lato; inflorescentia abbreviata, uniflora; pedunculo filiformi; sepalo postico ovato, apice longissime caudato, 3-nervio, margine ciliato; lamina 5–6 mm. longa, 4 mm. lata, cauda usque ad 2 cm. longa; sepalis lateralibus connatis, apice aristato-bifidis, lamina ovata, valde concava, 3-nervia, margine eciliata, 5–7 mm. longa, 6 mm. lata; cauda 10–17 mm. longa; petalis leviter obliquis, ovatis, 3-nerviis, apice longissime caudatis, margine ciliatis, sepalis aequilongis; labello e cuneata basi transverse rhombeo, subacuto cum apiculo late triangulo in medio, margine anteriore denticulato, 2–2.5 mm. longo, 3–4 mm. lato; disco 3-nervio, supra basin callo incrassato, triangulo ornato; columna brevissima, crassa, 1 mm. longa; brachiis inaequaliter bidentatis.

Costa Rica: Province of Heredia, Cerros de Zurqui, northeast of San Isidro. Coll. *P. C. Standley & J. Valerio No. 50759*, type (AMES, US); Cerro de las Caricias, north of San Isidro. Coll. *P. C. Standley & J. Valerio No. 52366* (AMES, US); ibidem *No. 52419* (AMES); Yerba Buena, San Isidro. Coll. *P. C. Standley & J. Valerio No. 50136* (US); sine loco speciali. Coll. *Endres No. 58* (W).

References

1. AMES, O. and SCHWEINFURTH, C. *In* Gleason, H. A. Botanical results of the Tyler-Duida Expedition. Bull. Torrey Botan. Club, 58 : 348. 1931.
2. GARAY, L. A. Contribution towards a monograph of the genus *Stelis* Sw. Can. J. Botany, 34 : 346-359. 1956.
3. KRÄNZLIN, F. Monographie der Gattungen *Masdevallia* etc. Fedde Rep. Beih. 34 : 9-11. 1925.
4. LINDLEY, J. Folia Orchidacea *Brachionidium*. London. J. Matthews, Covent Garden. 1. 1859.
5. REICHENBACH, H. G. Orchides. Walper's Ann. Botan. Systematicae, 6. 1863.
6. ROLFE, R. A. New Orchids: Decade 4. Kew Bull. Royal Botan. Gardens, 4. 1893.
7. ROLFE, R. A. and BROWN, N. E. *et al.* Report on two botanical collections made by Messrs. F. V. McConnell and J. J. Quelch at Mount Roraima in British Guiana. Trans. Linnean Soc. London, Ser. 2, Botany, 6 : 59. 1901.
8. SCHLECHTER, R. Systema Orchidacearum. Notizbl. Bot. Gart. u. Mus. Berlin-Dahlem 9, No. 88 : 563-691. 1926.
9. WILLIAMS, L. O. Orchid Studies XII. Botan. Museum Leaflets, Harvard Univ. 8 : 141-152. 1940.
10. WILLIAMS, L. O. The validity of the genus *Schomburgkia*. Darwiniana, 5 : 74-77. 1941.

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